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## **Environmental Risk Assessments Based on Bone Marrow Cell Kinetic**

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## SUMMARY

Consensus principles from radiation biology were used to describe a generic set of nonlinear, first-order differential equations for modeling of toxicity-induced compensatory cell kinetics following protracted irradiations. Cytological processes are analyzed in terms of sublethal injury, repair, direct killing, killing of cells with unrepaired sublethal injury, and repopulation. This cellular model was linked to a probit model of hematopoietic mortality that describes death from infection and/or hemorrhage between ~5 and 30 days. Mortality data from 27 experiments with 851 dose-response groups, in which doses were protracted by rate and/or fractionation, were used to simultaneously estimate all rate constants by maximum-likelihood methods. Data used represented 18,940 test animals distributed according to: (mice, 12,827); (rats, 2,925); (sheep, 1,676); (swine, 829); (dogs, 479); and (burros, 204). Although a long-term, repopulating hematopoietic stem cell is ancestral to all lineages needed to restore normal homeostasis, the dose-response data from the protracted irradiations indicate clearly that the particular lineage that is "critical" to hematopoietic recovery does not resemble stem-like cells with regard to radiosensitivity and repopulation rates. Instead, the weakest link in the chain of hematopoiesis was found to have an intrinsic radioresistance equal to or greater than stromal cells and to repopulate at the same rates. Model validation has been achieved by predicting the  $LD_{50}$  and/or fractional group mortality in 38 protracted-dose experiments (rats and mice) that were not used in the fitting of model coefficients. Models of risk for acute hematopoietic mortality and malignant cancers have been developed on the basis of compensatory marrow cell kinetics. This report briefly describes those models and provides an executable file of a User-Friendly Personal-Computer program referred to as MarCell (for marrow cell).

## PREFACE

Work reported in this report reflects major technical and administrative contributions by R.W. Young, Major Robert A. Kehlet, and Major Carl A. Curling. Additional contributions were provided by Lieutenant Colonel John Bliss, Sheldon Levin, George Anno, Gene McCellan, Rodney Withers, and John Ainsworth.

A User-Friendly, Menu-Driven executable file for a DOS-based personal computer is included with this report. Load and use the code with the following steps:

(in DOS)

step 1. md marcell

step 2. cd marcell

step 3. copy a:\\*.\* c:\\*.\*

Note--steps 1-3 load the code into the c:\marcell directory.

step 4. cd marcell (unnecessary after loading the code but required for successive runs).

step 5. marcel41

After step 5, the code prompts the user for menu choices.

# CONVERSION TABLE

Conversion factors for U.S. Customary to metric (SI) units of measurement.

MULTIPLY TO GET  $\longleftrightarrow$  BY TO GET  
TO GET  $\longleftrightarrow$  BY DIVIDE

angstrom	1.000 000 X E -10	meters (m)
atmosphere (normal)	1.013 25 X E +2	kilo pascal (kPa)
bar	1.000 000 X E +2	kilo pascal (kPa)
barn	1.000 000 X E -28	meter <sup>2</sup> (m <sup>2</sup> )
British thermal unit (thermochemical)	1.054 350 X E +3	joule (J)
calorie (thermochemical)	4.184 000	joule (J)
cal (thermochemical/cm <sup>2</sup> )	4.184 000 X E -2	mega joule/m <sup>2</sup> (MJ/m <sup>2</sup> )
curie	3.700 000 X E +1	*giga becquerel (GBq)
degree (angle)	1.745 329 X E -2	radian (rad)
degree Fahrenheit	$t_k = (t^{\circ}f + 459.67)/1.8$	degree kelvin (K)
electron volt	1.602 19 X E -19	joule (J)
erg	1.000 000 X E -7	joule (J)
erg/second	1.000 000 X E -7	watt (W)
foot	3.048 000 X E -1	meter (m)
foot-pound-force	1.355 818	joule (J)
gallon (U.S. liquid)	3.785 412 X E -3	meter <sup>3</sup> (m <sup>3</sup> )
inch	2.540 000 X E -2	meter (m)
jerk	1.000 000 X E +9	joule (J)
joule/kilogram (J/kg) radiation dose absorbed	1.000 000	Gray (Gy)
kilotons	4.183	terajoules
kip (1000 lbf)	4.448 222 X E +3	newton (N)
kip/inch <sup>2</sup> (ksi)	6.894 757 X E +3	kilo pascal (kPa)
ktap	1.000 000 X E +2	newton-second/m <sup>2</sup> (N-s/m <sup>2</sup> )
micron	1.000 000 X E -6	meter (m)
mil	2.540 000 X E -5	meter (m)
mile (international)	1.609 344 X E +3	meter (m)
ounce	2.834 952 X E -2	kilogram (kg)
pound-force (lbs avoirdupois)	4.448 222	newton (N)
pound-force inch	1.129 848 X E -1	newton-meter (N·m)
pound-force/inch	1.751 268 X E +2	newton/meter (N/m)
pound-force/foot <sup>2</sup>	4.788 026 X E -2	kilo pascal (kPa)
pound-force/inch <sup>2</sup> (psi)	6.894 757	kilo pascal (kPa)
pound-mass (lbm avoirdupois)	4.535 924 X E -1	kilogram (kg)
pound-mass-foot <sup>2</sup> (moment of inertia)	4.214 011 X E -2	kilogram-meter <sup>2</sup> (kg·m <sup>2</sup> )
pound-mass/foot <sup>3</sup>	1.601 846 X E +1	kilogram/meter <sup>3</sup> (kg/m <sup>3</sup> )
rad (radiation dose absorbed)	1.000 000 X E -2	**Gray (Gy)
roentgen	2.579 760 X E -4	coulomb/kilogram (C/kg)
shake	1.000 000 X E -8	second (s)
slug	1.459 390 X E +1	kilogram (kg)
torr (mm Hg, 0° C)	1.333 22 X E -1	kilo pascal (kPa)

\*The becquerel (Bq) is the SI unit of radioactivity; 1 Bq = 1 event/s.

\*\*The Gray (GY) is the SI unit of absorbed radiation.

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## SECTION 1

### INTRODUCTION

An editorial in *The American Statistician* by (Ehrenberg 1990), derived from experiences with business and marketing, insightfully describes a belief that analysis of many sets of data (MSOD) "*seems to be the only way in which we can produce results that are generalizable, lawlike, and predictable--which in fact hold for many sets of data . . . our concern will be with deciding what the main effect is quantitatively, how to model it, how consistent it is, under what different conditions it does or does not occur, why it arises, how it links up with other findings, and how it can be used in practical applications and/or in the development of theory.*" Although we have used such practices for nearly 20 years--in carcinogenic risk assessments, mathematical models of acute lethality, and marrow cell kinetics underlying radiation-induced hematopoiesis--we did not attempt to communicate those generalized ideas outside our particular areas of interest nor have we stated the essential ideas so compactly.

For mathematical models of dose-response effects, historically there has been a near-total reliance upon finding a simple equation that will approximate a single set of experimental data (SSOD) when the numerical constants are fitted appropriately. Fits to other data sets, from similar experimental protocols require additional statistical justification that the model is acceptable and new fitted parameters. Although continued use of the same functional form usually produces some attempt to establish a biological interpretation of the underlying effects (i.e., a conceptual model), in general, such interpretations usually have no fundamental validity and ignore far more important biological factors than the few they are hypothesized to approximate--even for those few factors, there is a pronounced lack of generality for protracted-, fractionated-, or variable-rate exposure protocols. Results from such exercises are without substantial validity outside the ranges of experimental conditions used and have no basis in reality when extrapolated in terms of dose, dose rate, or test species/strain used.

The general domain of biologically-based or conceptual models bifurcates into additional basic approaches. One pathway involves assumptions, either direct or indirect, that the important processes are known in terms of specific molecular/cellular effects and simple factors and descriptive models can be written accordingly. When indirect assumptions are involved, it is often overlooked that the conclusions obtained from experiment-by-experiment evaluations of the models are mandated either by the constraints of the

model or by limitations of the particular experiment used for estimation of parameters. Subtle, indirect assumptions have the hazard of going unrecognized, perhaps even to the experimentalists themselves.

Our approach formulates generalized dose-response models in terms of generic processes. Those generic processes may be: in terms of molecular effects; from a cell kinetics perspective; and descriptive of local and systemic reactions that may act through cell-to-cell and/or humoral mediated effects involving cytokines. The dosing schedules used for benzene experiments do not reflect adequate protocol-dependent variability to permit execution of the MSOD approach to a degree that provides informative insight into underlying biological mechanisms. In contrast, historical data from radiation biology do reflect those needed variations in experimental design. Those variations can be found at the molecular, cellular, organ, and organism levels, and all of those structural tiers have been considered to various degrees in model conceptualization, coefficient estimation, and model validation in our previous publications on radiation-induced hematopoiesis as listed in Table 1-1. Because our maximum likelihood estimations (MLE) have relied only upon lethality data from both prompt and protracted irradiations, those experiments serve as the data base used to evaluate the generic model in terms of cells "critical" to hematopoietic recovery. The data base used to evaluate the model for photon radiations is summarized in Figure 1-1.

Following is a brief description of how we have formulated a generic model for cell kinetics associated with radiation-induced hematopoiesis and how MSODs can be used to generalize the model and provide strong insight into the fundamental underlying mechanisms. Specifically, our intent was to use dogmatic terms and factors (or, as a minimum condition of acceptance those common to expert consensus) to approximate generic processes associated with marrow cell kinetics underlying acute lethality. Next, maximum-likelihood estimation methods were used to evaluate the numerical parameters of the models and their associated confidence bounds. This approach provides no direct cause-effect proof that the biologically-based model is indeed correct in all details but--because enormous sets of data, reflecting wide ranges of variability, can be fitted by a common set of evaluated parameters that are consistent with specific biological rate constants--it is obvious that the model is substantially correct in behavior and provides hypotheses that in turn may be validated or modified by further refinement of experimental design considerations. The result of that effort is summarized in Figure 1-2. In addition, we found it desirable to evaluate and test a cell kinetics model formulated in terms of those same non-specific damage, repair, and repopulation processes as derived from CFU-S experiments in contrast to the parallel evaluation made from the generic model and animal lethality data--i.e., the underlying dependence on "critical" cells is

not restricted to stem or CFU-S types of cells. That method is illustrated by the diagram shown in Figure 1-3. As indicated by Table 1-2, the conceptual and mathematical models, used for ionizing radiations, should also be relevant to radiomimetic chemicals such as benzene.

*Assumption: Acute Lethality Derives from Cytopenia of a "Critical" Bone-Marrow Cell:* When animals are irradiated by acute protocols, death from infection and/or hemorrhage may occur between about 5 and 30 days post-irradiation. The frequency of death can be described by a probit distribution function with fitted parameters comprised of the  $LD_{50}$  and slope (i.e., slope =  $\sigma^{-1}$  which is the inverse standard deviation of the frequency distribution). The  $LD_{50}$  and  $\sigma$  may be for the particular radiation field of interest or for a standard or reference radiation if there is a realistic way of modeling the underlying degree of cytopenia from the exposure of interest and converting that level of effect back to an equivalent reference dose of the standard radiation associated with the  $LD_{50}$  and  $\sigma$  estimates. Depression of neutrophils and platelets are accepted as the proximate cause of death, but the contributing cause of death could be either the terminally differentiated cells themselves; ancestral cells; or ancestral-dependent lineages upstream in the direction of the undifferentiated pluripotent stem cells as illustrated in Figure 1-4. For generality, the weakest link (i.e., lineage) was treated generically and guided by MLE evaluations in contrast to more restrictive assumptions. One major advantage of this approach is that only one ( $LD_{50}$ ,  $\sigma$ ) combination was required for a complex experiment involving different: dose rates, exposure protocols, radiation sources, etc. In short, only changes with respect to the strain, species, cage care, and conditions of observation required additional  $LD_{50}$  and  $\sigma$  values. One experiment in the analysis was comprised of 26 different  $LD_{50}$  protocols but all were consistent with a common  $LD_{50}$  and  $\sigma$  associated with an "equivalent prompt dose."

*Generic Cellular Model Evaluated from Animal Lethality Data:* Theory underlying the model and likelihood analysis have been described in the publications cited in Table 1-1. In the mathematical model, cells are compartmentalized into normal (N), injured (I), and killed (K) populations. Processes by which cells move among those populations are modeled by first-order, nonlinear equations. In an arbitrary volume of marrow, we call the numbers of normal, injured and killed cells  $n_N$ ,  $n_I$  and  $n_K$ , respectively. Initial conditions are  $n_N = n_0$  (normal before exposure),  $n_I = 0$  (no injury before exposure) and  $n_K = 0$

(no killing before exposure). The  $n_o$  need not be estimated because only ratios of  $n_N$ ,  $n_I$  and  $n_K$  relative to  $n_o$  are used. The cellular component of the model is

$$n'_N = -\lambda_{NI}D'n_N - \lambda_{NK}D'n_N + \lambda_{IN}F_{IN}n_I + \lambda_{NN}MF_{NN}n_N \quad (1.1)$$

$$n'_I = -\lambda_{IK}D'n_I - \lambda_{IN}F_{IN}n_I + \lambda_{NI}D'n_N \quad (1.2)$$

$$n'_K = \lambda_{NK}D'n_N + \lambda_{IK}D'n_I \quad (1.3)$$

$\lambda$ s are rate constants that mediate movements of cells from normal or injured states as indicated by the first subscript to the state indicated by the second subscript.  $D$  is dose given uniformly to marrow, and prime (') denotes the derivative of a cell count or dose (i.e., dose rate) with respect to time. Factors and terms of Equations (1.1) to (1.3) are given in Table 1-3. Model constants representative of stroma are given in Table 1-4 and constants for hematopoietic stem cells are given in Table 1-5.

*Hematopoiesis Model Evaluated from CFU-S Data:* The same functional form based on cellular damage, repair, and repopulation was evaluated from experimental studies on CFU-S cells. Damage constants were estimated from dose-rate data of (Puro and Clark 1972). The proliferation constant was estimated from an analysis of published values obtained from an extensive literature review. The repair constant was taken from the evaluation described above for the lethality data base, but an additional normalization was required to adjust for the shorter cycle time of stem/CFU-S cells in contrast to the longer cycle for the "critical" cells.

The two models of marrow cell kinetics involve (a) cells that are "critical" to compensatory hematopoiesis with parameters estimated from MLE analysis of animal mortality data and (b) CFU-S type stem cells with parameters fitted from in vivo and in vitro cell-survival studies. Both models seem to perform remarkably well according to the foundations of their evaluations. Clearly the point estimates and confidence intervals on estimated coefficients indicate that the two cellular models are distinct and do not merely provide dual estimates for a common lineage.

*Model validation:* The 38 experiments used to validate the model (selected as described above) typically reported only LD<sub>50</sub> values without giving the actual dose-response data. Although these studies were not useful for un-biased estimation of model constants, they do provide independent tests for model validation. The 12 doses rate studies ranged from 0.08 to 474 r/min and the 26 fractionation studies contained fractionations from 154 to 700 r given over periods ranging from hours to 8 weeks. Although the conversion of a protracted protocol to its prompt dose equivalence is cell-lineage dependent, that

conversion for very simple fractionated protocols will generally produce numerically similar estimates of the EPD and it is not clear which lineage better explains the biology underlying acute mortality. In contrast, complex fractionation experiments and low dose-rate studies are sensitive to lineage-specific effects and result in different estimates for the EPD. These lineage-dependent EPD estimates clearly favor either a stem or a stromal cell type model. As seen in Figure 1-5, the results overwhelmingly indicate that a radioresistant, slowly repopulating cell is far more consistent with the biological processes underlying acute mortality, otherwise at least 50% of the distribution should be below the abscissa value of 1.0.

An exhaustive literature review was made to compile a data base on the radiosensitivity and repopulation rates for human leukemia and lymphoma cells. From that data base, model rate constants for cell kinetics were estimated as listed in Table 1-6. Description of that task will be given in a later section of this report.

A student training grant, Sponsored by the U.S. Department of Energy at Oak Ridge National Laboratory under Contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. and The Great Lakes College Association was obtained for Jafar Syed Hasan. That effort was used to develop User-Friendly, personal computer input and output interfaces so that stromal cell, hematopoietic stem cell, and malignant leukemia and lymphoma cell kinetics calculations could be conducted easily by any interested user with a DOS-based computer. Executable files of the cell-kinetics program, in addition to the User-Friendly I/O interface routines are included in the diskette provided with this report. Sample screens from the program are shown in Section 2.

Table 1-1. List of journal publications on the marrow stromal and hematopoietic stem cell kinetics models for risks from protracted irradiations

**Experimental Hematology**

Marrow Stroma and Myelopoiesis. 21(6):Front Cover, 1993.

A Cell-Kinetics Model of Radiation-Induced Myelopoiesis. 21:816-822, 1993.

Do Stem or Stromal Cells Control Hematopoietic Recovery after Irradiation? 22:(Correspondence) 1994.

Response to the Letter of A.C.C. Ruifrok and H.D. Thames. 22:(Correspondence) 536-538, 1994.

**Health Physics**

Dose-Rate RBE Factors for Photons: Hematopoietic Syndrome in Humans vs. Stromal Cell Cytopenia. 67:495-508, 1994.

Bone Marrow Equivalent Prompt Dose from two Common Fallout Scenarios. 67:183-186, 1994.

Some Effects of Radiation Dosimetry Errors on an Estimated Dose-Response Relationship. 56:219-222, 1989.

**International Journal of Radiation Biology**

Hematopoietic Death of Unprotected Man from Photon Irradiations: Statistical Modeling from Animal Experiments. 55:445-461, 1989.

A Comparison of Dose-Response Models for Death from Hematological Depression in Different Species. 53:439-456, 1988.

**International Journal of Radiation Oncology, Biology, Physics**

Mathematical Models of Marrow Cell Kinetics: Differential Effects of Protracted Irradiations on Stromal and Stem Cells in Mice. 26:817-830, 1993.

**Radiation Research**

A Cell Kinetics Model of Radiation-Induced Myelopoiesis: Rate Coefficient Estimates for Mouse, Rat, Sheep, Swine, Dog, and Burro Irradiated by Photons. 135:320-331, 1993.

A Mathematical Model for Radiation-Induced Myelopoiesis. 128:258-266.

Estimation of Coefficients in a Model of Radiation-Induced Myelopoiesis from Mortality Data for Mice Following X-Ray Exposure. 128:267-275, 1991.

Response to the Letter of S.A. Roberts and J.H. Hendry. 138:(Correspondence) 303-305, 1994.

**In Review for Journal Publication**

Mathematical Models of Marrow Cell Kinetics: Neutron RBEs for Cytopenia and Repopulation of Stromal and Stem Cells.

Additional Perspective on Protracted Irradiation Schedules, Murine LD<sub>50%/30 day</sub> Experiments, Hematopoiesis, and the Cytokine-Producing Microenvironment of the Marrow Repopulating Cells.

Modeling Marrow Damage from Response Data: Morphallaxis from Radiation Biology to Benzene Toxicity.

Table 1-2. Summary of bioassays and test conditions that have been used to measure the toxicity of benzene.

Tests	Organisms	Routes	Cell Types
Chromosome aberrations	bacteria	eye	bone marrow
DNA damage	cat	inhalation	embryo
DNA inhibition	dog	intraperitoneal	fibroblast
DNA unscheduled synthesis	drosophila	intravenous	Hela
Dominant lethal	frog	oral	leukocyte
Gene conversion & mitotic recombination	grasshopper	parenteral	liver
Micronucleus	guinea pig	skin	lung
Microsomal mutagenicity	hamster	subcutaneous	lymphocyte
Mutation in somatic mammalian cells	human		ovary
Mutation in microorganisms (w/o S9)	molds		
Mutation in microorganisms (S9)	mouse		
Oncogenic transformation	non-mammals		
Sex chromosome loss and disjunction	rabbit		
Specific locus	rat		
Sister chromatid exchanges	yeast		



**Table 1-3.** Summary of non-specific processes used to write a generic cell-kinetics model for damage, repair, and repopulation as a consequence of protracted exposure to ionizing radiations. Animal lethality studies were analyzed by maximum likelihood estimation (MLE) techniques in order to estimate the values for cellular rate constants for processes of sublethal injury, repair, direct killing, killing of cells having unrepaired sublethal injury, and compensatory repopulation. Subscripts are: I = sublethal injury; N = normal; K = kill; 0 = time at zero condition where all cells are phenotypically normal ( $n_N = n_0 = 1$ ), the population of killed cells  $N_K = 0$ ; and the population of injured cells  $n_I = 0$ . Because the model permits a cell to be in one of three phenotypically distinct states, the subscripts on the cellular rate constants ( $\lambda$ ) indicate movement of cells from one compartment to another, e.g.,  $\lambda_{NI}$  indicates repair to phenotypically normal function from an injured state.

Process:	Term:	Definition:
Sublethal injury:	$\lambda_{NI}D'n_N$	$\lambda_{NI}$ = MLE constant (cells/Gy) $D'(t)$ = dose rate (Gy/min) $n_N$ = cells at risk of sublethal injury
Repair of Sublethal Injury:	$\lambda_{IN}F_{IN}n_I$	$\lambda_{IN}$ = MLE constant (cells/min) $n_I$ = cells that can undergo repair of sublethal injury $F_{IN}$ = rate modifying factor taken to be in the range of [1,2] and set at $1 + (n_0 - n_N - n_I)/n_0$ from fits to experimental data on the mitotic cycle
Direct Killing of Cells:	$\lambda_{NK}D'(t)n_N$	$\lambda_{NK}$ = MLE constant (cells/Gy)
Indirect Killing of Cells:	$\lambda_{IK}D'(t)n_I$	$\lambda_{IK}$ = MLE constant (cells/Gy) $n_I$ = cells that have sublethal injury and can be killed by indirect processes
Compensatory Repopulation:	$\lambda_{NN}MF_{NN}n_N$	$\lambda_{NN}$ = MLE constant (cells/min) $n_N$ = phenotypically normal cells that can undergo mitosis $F_{NN} = (n_0 - n_N - n_I)/n_0 \bullet F_{IN}$ $(n_0 - n_N - n_I)/n_0$ controls proliferation rate; increases with cytotoxicity and stops at homeostasis  M = Dirac delta function to turn on/off mitotic delay = 0, when time (hr) > accumulated dose (Gy), and = 1, when accumulated dose (Gy) $\geq$ time (hr)

\*\*\*\*\*

#### MLE Values for Numerical Constants<sup>a</sup>

$\lambda_{NI} = 0.38$  to  $0.77 \text{ Gy}^{-1}$ ;  $\lambda_{NK} = 0.12$  to  $0.24 \text{ Gy}^{-1}$ ;  $\lambda_{IK} = 0.32$  to  $0.50 \text{ Gy}^{-1}$ ; and  $\lambda_{IN} = 0.022 \text{ min}^{-1}$ .

Experimental studies have typically found that visible chromosomal aberrations may occur with a frequency of about 0.1 CA/Gy, which seems to be reasonably consistent with the composite action of  $\lambda_{NI}$ ,  $\lambda_{NK}$ ,  $\lambda_{IK}$ , and  $\lambda_{IN}$ . For  $\lambda_{IN}$  the repair half-times would range from 15 to 31 minutes depending upon the length of the mitotic cycle. For comparison, published half-times for repair of DNA-DSBs typically range from about 10 min to 2 hours which includes estimates for both fast and slow repair in various lineages of cells from different species and tissues.

$\lambda_{NN}$  (in units of  $\text{min}^{-1}$ ) values of  $8.26 \times 10^{-5}$  (mouse);  $4.54 \times 10^{-5}$  (rat);  $4.23 \times 10^{-5}$  (sheep);  $1.65 \times 10^{-4}$  (swine); and  $8.89 \times 10^{-5}$  (dog) give estimates of the doubling time (in units of hour) for compensatory repopulation as follows:

Species:	$T_D$ ( for $LD_{10}$ to $LD_{50}$ )	$T_D$ (for Therapeutic Fractions)	$T_D$ (for 0.25 Gy <sup>b</sup> )
Swine	35	70	9 wk
Dog	65	130	14 wk
Mouse	70	140	14 wk
Rat	130	260	26 wk
Sheep	140	280	36 wk

<sup>a</sup> Ranges listed include variations assigned for species-specific DNA and radiation quality for 100 kVp X, 250 kVp X, <sup>137</sup>Cs, and <sup>60</sup>Co photons. Variations due to randomness and/or analysis are not estimated in this paper.

<sup>b</sup> 0.25 Gy has been used historically as a maximum for radiation workers responding to a criticality accident. In addition, the estimates given in column 4 would approximate the final degree of healing following more serious, even near fatal injury.

Table 1-4. Rate constants for radioresistant cells determined to be "critical" to myelopoiesis from maximum-likelihood analysis of mortality data. The coefficients shown for mixed-field neutron and gamma radiations do not contain adjustments to account for possible hardening or softening of the radiation spectrum due to reactor shields or moderators.

CELL PROCESS	UNITS	RADIATION <sup>a</sup>	MOUSE	RAT	DOG	SHEEP	SWINE	BURRO	(MAN)
Compensatory Repopulation	(min <sup>-1</sup> )	All	0.000083	0.000045	0.000089	0.000042	0.00016	< 10 <sup>-4</sup>	0.000083
Repair of Sublethal	(min <sup>-1</sup> )	X & γ RAYS	0.022	0.022	0.022	0.022	0.022	0.022	0.022
		NEUTRONS	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		n:γ = 1:1	0.022	0.022	0.022	0.022	0.022	0.022	0.022
		n:γ = 5:1	0.022	0.022	0.022	0.022	0.022	0.022	0.022
		n:γ = 30:1	0.022	0.022	0.022	0.022	0.022	0.022	0.022
DNA per cell (relative to mouse)			100	98	91	76	76	87	92.6
Sublethal <sup>b</sup> Injury	(cGy <sup>-1</sup> )	Tritium beta	0.0083	0.0081	0.0074	0.0062	0.0062	0.0072	0.0077
		100 kVp X	0.0077	0.0076	0.0067	0.0059	0.0059	0.0067	0.0071
		250 kVp X	0.0068	0.0066	0.0061	0.0051	0.0051	0.0059	0.0063
		<sup>137</sup> Cs	0.0060	0.0058	0.0054	0.0045	0.0045	0.0052	0.0055
		<sup>60</sup> Co	0.0050	0.0049	0.0045	0.0038	0.0038	0.0044	0.0046
		2 MeV electrons	0.0044	0.0043	0.0040	0.0033	0.0033	0.0038	0.0041
		22 MV X	0.0044	0.0042	0.0030	0.0033	0.0033	0.0038	0.0040
		Fission N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		Fusion N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		n:γ = 1:1	0.0025	0.0024	0.0023	0.0019	0.0019	0.0022	0.0023
		n:γ = 5:1	0.00083	0.00082	0.00076	0.00063	0.00063	0.00072	0.00077
		n:γ = 30:1	0.00016	0.00016	0.00015	0.00012	0.00012	0.00014	0.00015
1-Hit <sup>b</sup> Killing	(cGy <sup>-1</sup> )	Tritium beta	0.0026	0.0026	0.0023	0.0020	0.0020	0.0022	0.0024
		100 kVp X	0.0024	0.0024	0.0022	0.0018	0.0018	0.0021	0.0022
		250 kVp X	0.0021	0.0021	0.0019	0.0016	0.0016	0.0018	0.0020
		<sup>137</sup> Cs	0.0018	0.0018	0.0017	0.0014	0.0014	0.0016	0.0017
		<sup>60</sup> Co	0.0016	0.0015	0.0014	0.0012	0.0012	0.0014	0.0014
		2 MeV electrons	0.0014	0.0014	0.0012	0.0010	0.0010	0.0012	0.0013
		22 MV X	0.0013	0.0013	0.0012	0.0010	0.0010	0.0012	0.0013
		Fission N	0.0067	0.0066	0.0061	0.0051	0.0051	0.0059	0.0062
		Fusion N	0.0050	0.0049	0.0045	0.0038	0.0038	0.0043	0.0046
		n:γ = 1:1	0.0042	0.0040	0.0038	0.0032	0.0032	0.0036	0.0038
		n:γ = 5:1	0.0059	0.0057	0.0053	0.0045	0.0045	0.0051	0.0054
		n:γ = 30:1	0.0066	0.0064	0.0060	0.0050	0.0050	0.0057	0.0061
Killing after <sup>c</sup> Sublethal Injury	(cGy <sup>-1</sup> )	Tritium beta	0.0054	0.0054	0.0054	0.0054	0.0054	0.0054	0.0054
		100 kVp X	0.0050	0.0050	0.0050	0.0050	0.0050	0.0050	0.0050
		250 kVp X	0.0044	0.0044	0.0044	0.0044	0.0044	0.0044	0.0044
		<sup>137</sup> Cs	0.0038	0.0038	0.0038	0.0038	0.0038	0.0038	0.0038
		<sup>60</sup> Co	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032	0.0032
		2 MeV electrons	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029	0.0029
		22 MV X	0.0028	0.0028	0.0028	0.0028	0.0028	0.0028	0.0028
		Fission N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		Fusion N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		n:γ = 1:1	0.0083	0.0085	0.0086	0.0085	0.0085	0.0084	0.0087
		n:γ = 5:1	0.012	0.012	0.012	0.012	0.012	0.012	0.012
		n:γ = 30:1	0.013	0.013	0.014	0.013	0.013	0.013	0.014

<sup>a</sup> Triga neutrons with neutron dose,  $D_N$ , to gamma dose,  $D_G$ , ratio of 1:1; 5:1; or 30:1.

<sup>b</sup> Composite rates estimated from  $[\lambda_{N+G}] = ([\lambda_N] \times D_N + [\lambda_G] \times D_G) / D$ , where  $D_N = D/2$ ,  $5D/6$ , or  $30D/31$  and  $D_G = (1 - D_N)$ .  $[\lambda_G]$  taken from <sup>60</sup>Co.

<sup>c</sup> The ratio of  $\lambda_{ik} / \lambda_{nk}$  for a particular photon radiation suggests that a "critical" cell having unrepaired sublethal injury may be more radiosensitive to killing than a normal cell--hence,

$$[\lambda_{ik}]_{N+G} = \{D_G \times [\lambda_{ik}]_G + D_N \times (\lambda_{ik}/\lambda_{nk})_G \times [\lambda_{nk}]_N\} / D_{N+G}.$$

Table 1-5. Cellular rate constants for CFU-S (or hematopoietic stem) cells. The coefficients shown for mixed-field neutron and gamma radiations do not contain adjustments to account for possible hardening or softening of the radiation spectrum due to reactor shields or moderators.

CELLULAR	UNITS	RADIATION <sup>a</sup>	MOUSE	RAT	DOG	SHEEP	SWINE	BURRO	(MAN)
Compensatory Repopulation	(min <sup>-1</sup> )	All	0.00022	0.00022	0.00022	0.00022	0.00022	0.00022	0.00022
Repair of Sublethal	(min <sup>-1</sup> )	X & γ RAYS	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		NEUTRONS	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		n:γ = 1:1	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		n:γ = 5:1	0.060	0.060	0.060	0.060	0.060	0.060	0.060
		n:γ = 30:1	0.060	0.060	0.060	0.060	0.060	0.060	0.060
DNA per cell (relative to mouse)			100	98	91	76	76	87	92.6
Sublethal <sup>b</sup> Injury	(cGy <sup>-1</sup> )	Tritium beta	0.013	0.013	0.012	0.010	0.010	0.012	0.012
		100 kVp X	0.013	0.013	0.012	0.0099	0.0099	0.011	0.012
		250 kVp X	0.011	0.011	0.010	0.0084	0.0084	0.0096	0.010
		<sup>137</sup> Cs	0.0097	0.0095	0.0088	0.0074	0.0074	0.0084	0.0090
		<sup>60</sup> Co	0.0081	0.0079	0.0074	0.0062	0.0062	0.0070	0.0075
		2 MeV electrons	0.0072	0.0072	0.0065	0.0055	0.0055	0.0062	0.0065
		22 MV X	0.0070	0.0070	0.0064	0.0054	0.0054	0.0061	0.0064
		Fission N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		Fusion N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		n:γ = 1:1	0.0040	0.0039	0.0037	0.0031	0.0031	0.0035	0.0037
		n:γ = 5:1	0.0014	0.0014	0.0013	0.0011	0.0011	0.0012	0.0013
		n:γ = 30:1	0.00026	0.00026	0.00024	0.00020	0.00020	0.00023	0.00024
1-Hit <sup>b</sup> Killing	(cGy <sup>-1</sup> )	Tritium beta	0.0048	0.0046	0.0043	0.0037	0.0037	0.0041	0.0044
		100 kVp X	0.0044	0.0043	0.0040	0.0033	0.0033	0.0038	0.0041
		250 kVp X	0.0039	0.0038	0.0035	0.0030	0.0030	0.0034	0.0036
		<sup>137</sup> Cs	0.0034	0.0033	0.0031	0.0026	0.0026	0.0030	0.0031
		<sup>60</sup> Co	0.0029	0.0028	0.0026	0.0022	0.0022	0.0025	0.0027
		2 MeV electrons	0.0025	0.0025	0.0023	0.0020	0.0020	0.0022	0.0023
		22 MV X	0.0025	0.0024	0.0022	0.0019	0.0019	0.0022	0.0023
		Fission N	0.022	0.022	0.020	0.017	0.017	0.019	0.020
		Fusion N	0.015	0.015	0.014	0.011	0.011	0.013	0.014
		n:γ = 1:1	0.012	0.012	0.011	0.0091	0.0091	0.010	0.011
		n:γ = 5:1	0.019	0.019	0.017	0.014	0.014	0.017	0.018
		n:γ = 30:1	0.021	0.021	0.019	0.016	0.016	0.018	0.019
Killing after <sup>c</sup> Sublethal Injury	(cGy <sup>-1</sup> )	tritium beta	0.16	0.16	0.16	0.16	0.16	0.16	0.16
		100 kVp X	0.13	0.13	0.13	0.13	0.13	0.13	0.13
		250 kVp X	0.11	0.11	0.11	0.11	0.11	0.11	0.11
		<sup>137</sup> Cs	0.097	0.097	0.097	0.097	0.097	0.097	0.097
		<sup>60</sup> Co	0.081	0.081	0.081	0.081	0.081	0.081	0.081
		2 MeV electrons	0.072	0.072	0.072	0.072	0.072	0.072	0.072
		22 MV X	0.070	0.070	0.070	0.070	0.070	0.070	0.070
		Fission N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		Fusion N	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000
		n:γ = 1:1	0.35	0.36	0.35	0.35	0.35	0.34	0.34
		n:γ = 5:1	0.53	0.54	0.53	0.54	0.52	0.51	0.51
		n:γ = 30:1	0.60	0.62	0.60	0.61	0.61	0.59	0.58

<sup>a</sup> Triga neutrons with neutron dose,  $D_N$ , to gamma dose,  $D_G$ , ratio of 1:1; 5:1; or 30:1.

<sup>b</sup> Composite rates estimated from  $[\lambda]_{N+G} = ([\lambda]_N \times D_N + [\lambda]_G \times D_G) / D$ , where  $D_N = D/2$ ,  $5D/6$ , or  $30D/31$  and  $D_G = (1 - D_N)$ .  $[\lambda]_G$  taken from <sup>60</sup>Co.

<sup>c</sup> The ratio of  $\lambda_{ik} / \lambda_{nk}$  for a particular photon radiation suggests that a CFU-S cell having unrepaired sublethal injury may be more radiosensitive to killing than a normal cell--hence,

$$[\lambda_{ik}]_{N+G} = \{D_G \times [\lambda_{ik}]_G + D_N \times (\lambda_{ik}/\lambda_{nk})_G \times [\lambda_{nk}]_N\} / D_{N+G}.$$

Table 1-6. Cellular rate constants for hematopoietic stem cells, four reference lineages of leukemia/lymphoma cells, and marrow stromal cells.

CELLULAR	UNITS	RADIATION	HSCs	M1 Cells	M2 Cells	M3 Cells	M4 Cells	Stromal Cells
<b>Compensatory Repopulation</b>	(min <sup>-1</sup> )	All	0.0022	<i>a</i>	<i>a</i>	<i>a</i>	<i>a</i>	0.000826
<b>Repair of Sublethal</b>	(min <sup>-1</sup> )	X & γ RAYS	0.060	0.075	0.048	0.028	0.024	0.0222
<b>Sublethal Injury</b>	(cGy <sup>-1</sup> )	Tritium beta	0.012	0.015	0.011	0.0085	0.0080	0.0077
		100 kVp X	0.012	0.013	0.010	0.0081	0.0077	0.0071
		250 kVp X	0.010	0.012	0.0091	0.0070	0.0067	0.0063
		<sup>137</sup> Cs	0.0090	0.010	0.0080	0.0062	0.0059	0.0055
		<sup>60</sup> Co	0.0075	0.0084	0.0067	0.0052	0.0050	0.0046
		2 MeV electrons	0.0065	0.0078	0.0059	0.0046	0.0044	0.0041
<b>1-Hit Killing</b>	(cGy <sup>-1</sup> )	22 MV X	0.0064	0.0077	0.0058	0.0045	0.0042	0.0040
		Tritium beta	0.0044	0.0050	0.0038	0.0028	0.0024	0.0024
		100 kVp X	0.0041	0.0047	0.0035	0.0026	0.0023	0.0022
		250 kVp X	0.0036	0.0041	0.0031	0.0023	0.0021	0.0020
		<sup>137</sup> Cs	0.0031	0.0036	0.0027	0.0020	0.0018	0.0017
		<sup>60</sup> Co	0.0027	0.0024	0.0023	0.0017	0.0015	0.0015
<b>Killing after Sublethal Injury</b>	(cGy <sup>-1</sup> )	2 MeV electrons	0.0023	0.0027	0.0020	0.0015	0.0013	0.0013
		22 MV X	0.0023	0.0026	0.0020	0.0015	0.0013	0.0013
		Tritium beta	0.13	0.20	0.098	0.032	0.0083	0.0054
		100 kVp X	0.13	0.18	0.091	0.030	0.0079	0.0050
		250 kVp X	0.11	0.16	0.080	0.026	0.0070	0.0044
		<sup>137</sup> Cs	0.097	0.14	0.070	0.023	0.0061	0.0039
<b>Sublethal Injury</b>	(cGy <sup>-1</sup> )	<sup>60</sup> Co	0.081	0.12	0.059	0.019	0.0051	0.0032
		2 MeV electrons	0.072	0.10	0.052	0.017	0.0045	0.0029
		22 MV X	0.070	0.10	0.051	0.017	0.0044	0.0028

*a* Proliferation constants will be estimated from cell cycle time or tumor doubling time given as input data--see the text.

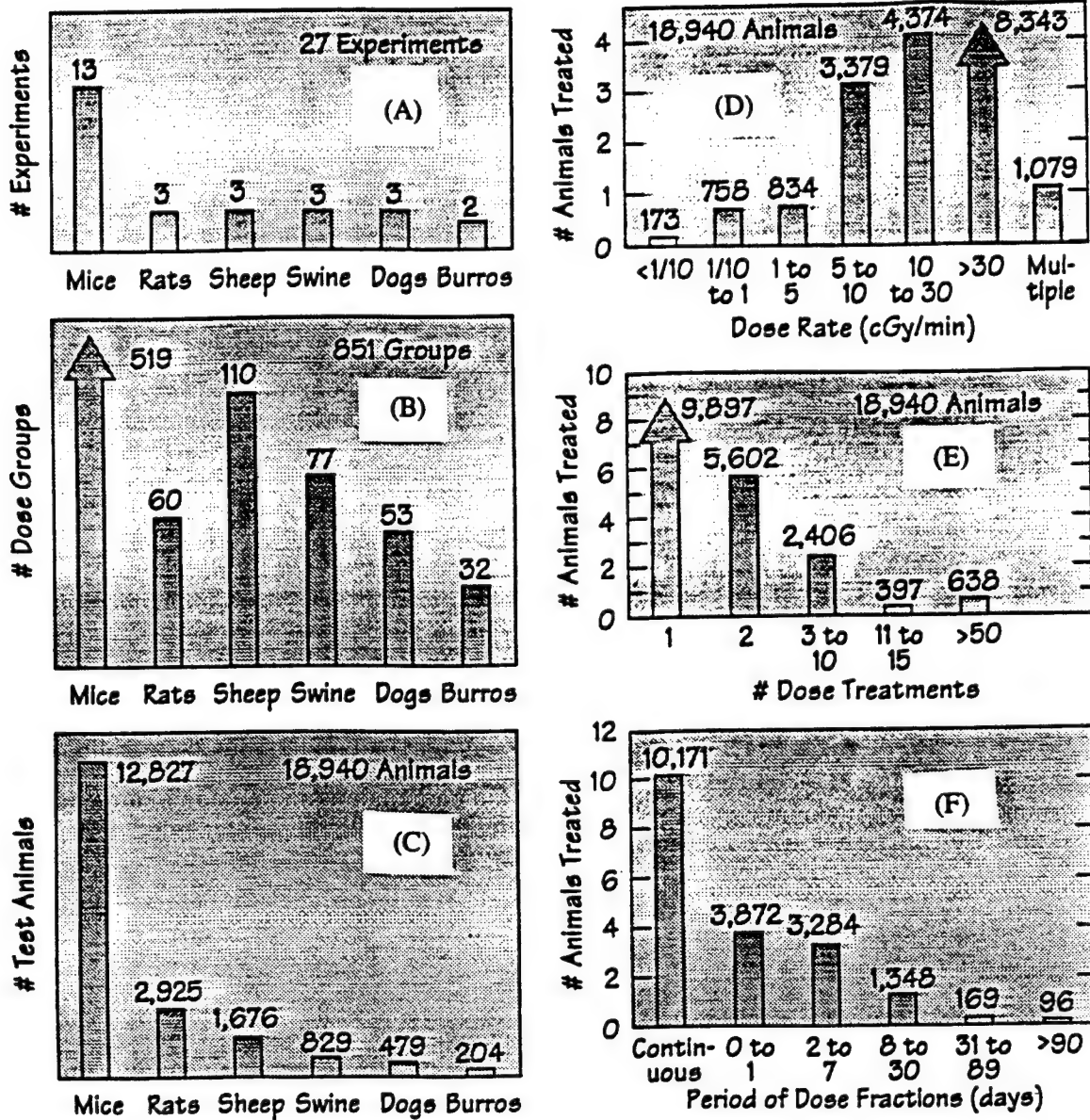


Figure 1-1. Summary of data base used to develop the photon cell-kinetics model for mortality. Panels include: (A) Number of experiments vs species. (B) Number of animal groups vs species. (C) Number of test animals vs species. (D) Number of test animals of all species vs dose rate. (E) number of animals treated vs number of dose fractions given. and (F) Number of animals vs the period of the treatment schedule.

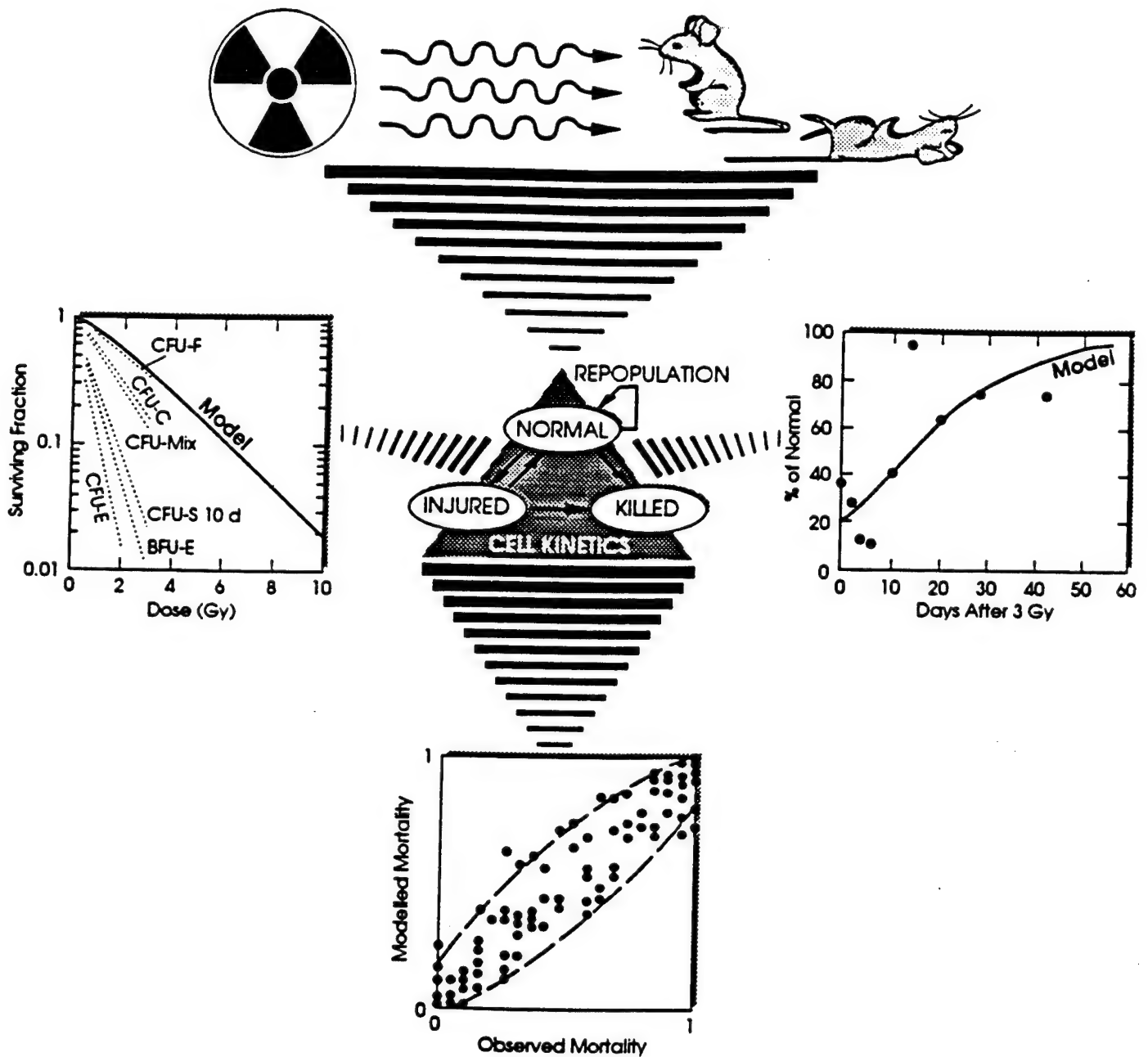


Figure 1-2. Illustration of how radiation protocol data, animal mortality data, and the cytokinetic model link together to produce a prompt dose cell survival curve and repopulation kinetics vs time post-exposure. This figure was featured on the cover of *Experimental Hematology*.

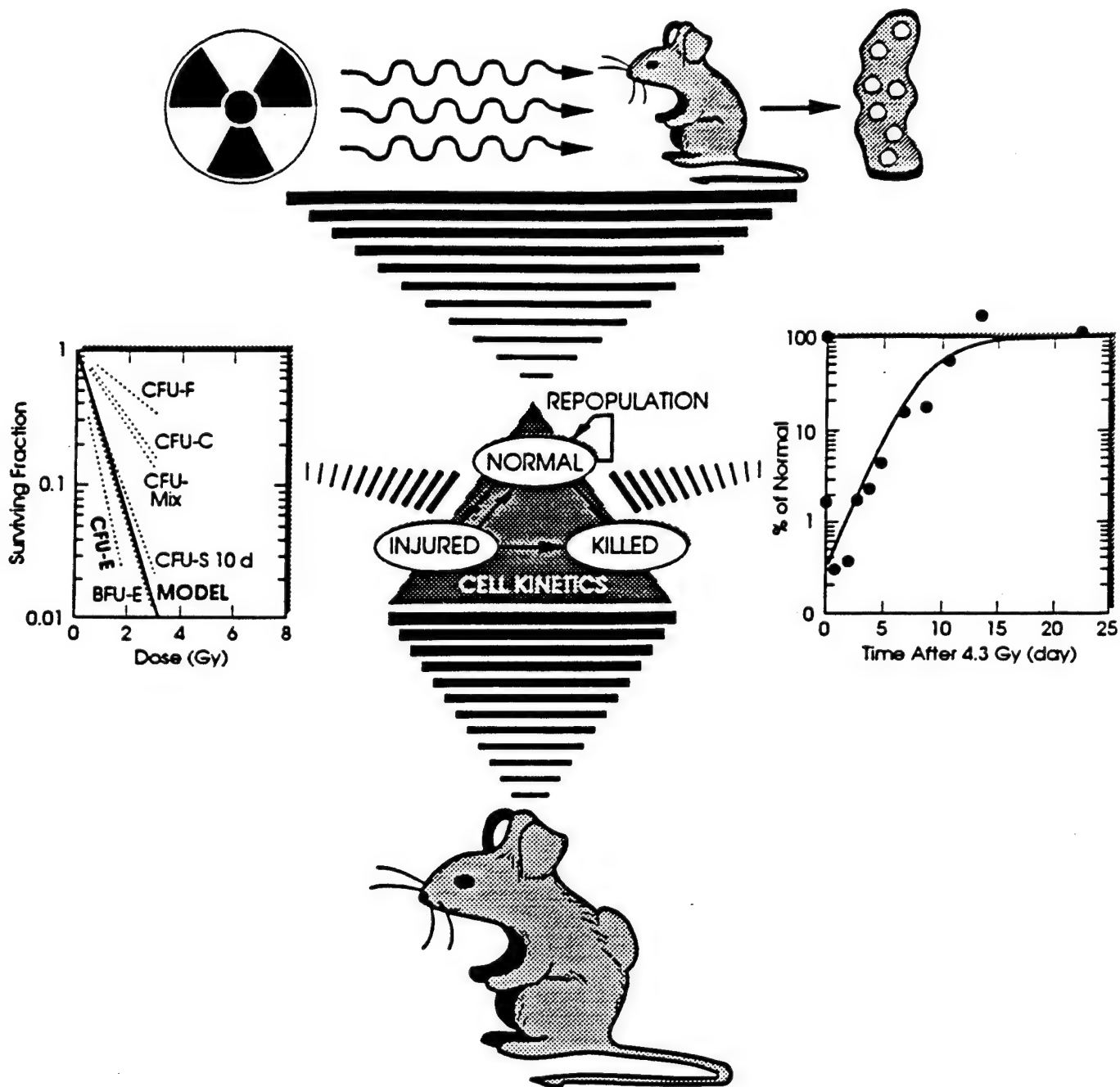


Figure 1-3. Illustration of how radiation protocol data, and cell-survival and repopulation data in the spleen cell colony bioassay were used to evaluate model constants for the hematopoietic stem cells. The evaluated model has the radiosensitivities and repopulation kinetics shown in the side panels and should be relevant to chronic risk assessments as illustrated in the panel at the bottom.

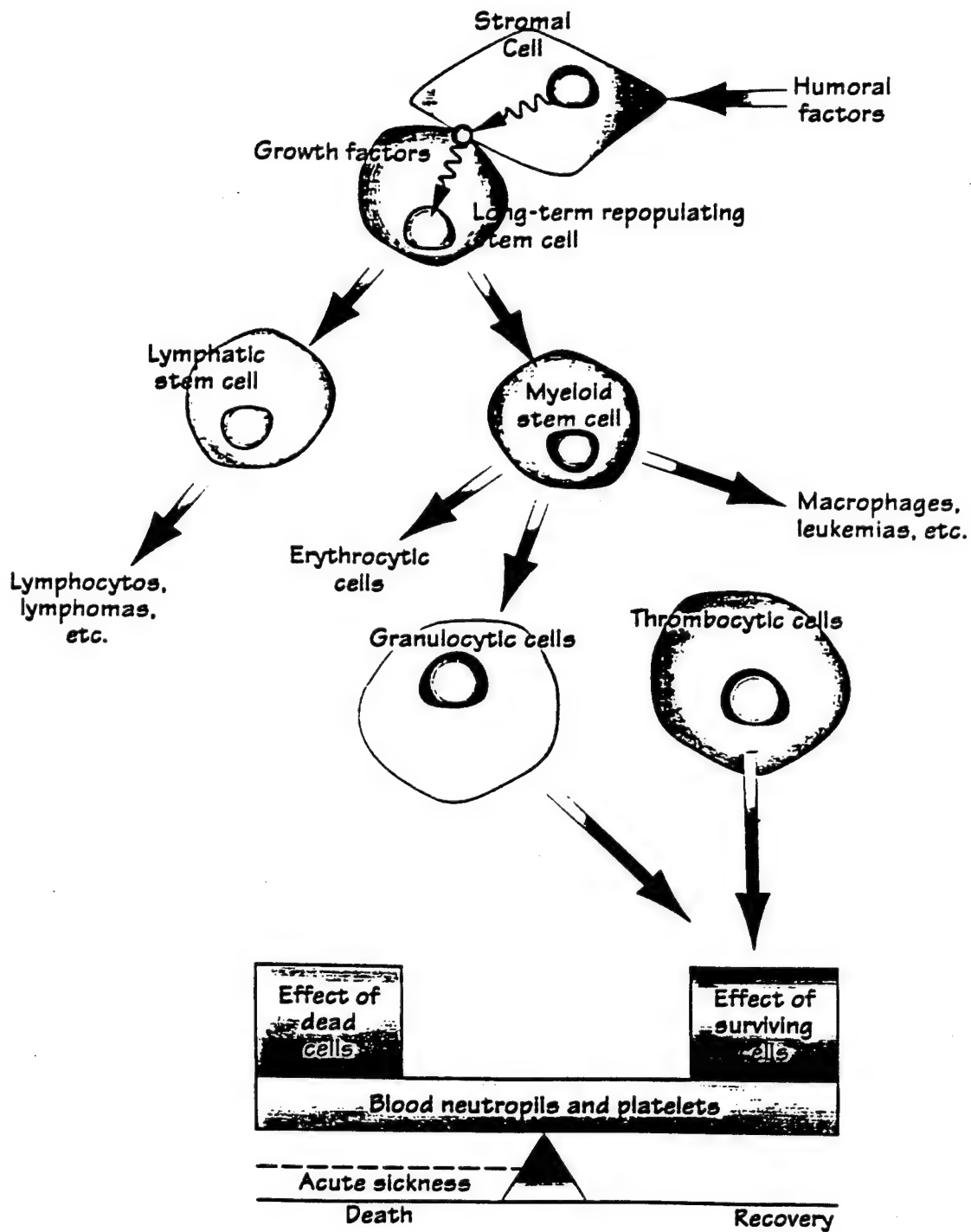


Figure 1-4. Illustration of cytokinetic processes underlying acute radiation mortality from hematopoietic depression and how the model balances the effect of cytopenia against death or recovery.



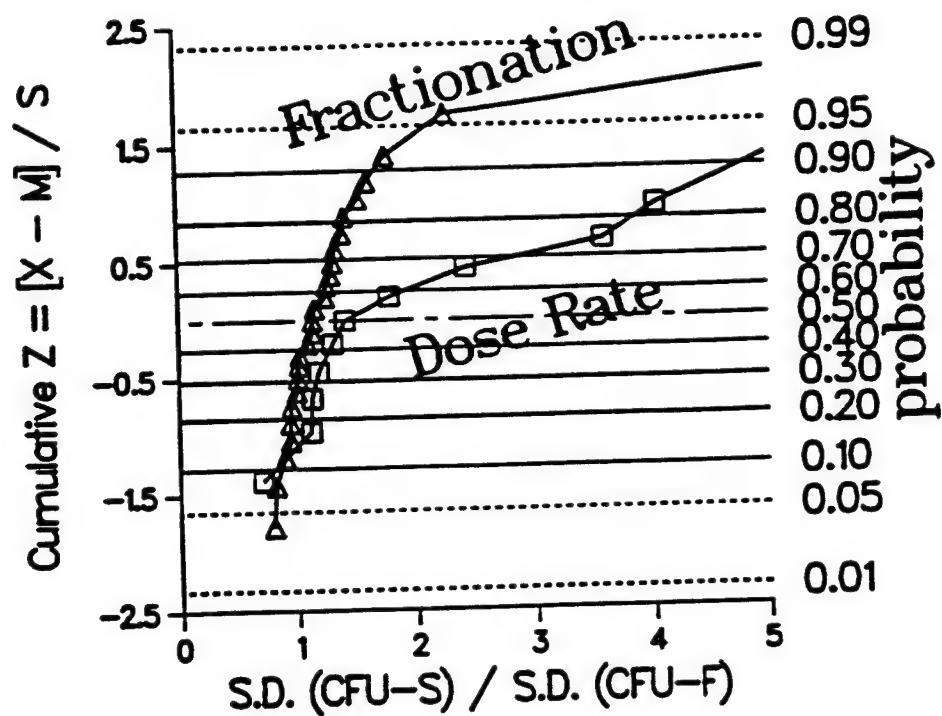


Figure 1-5. Comparisons of how stromal cell kinetics improves the prediction of acute mortality over results from the use of a stem cell model. Fractionated and dose-rate protocols are given in Table 6-1.

## SECTION 2

### MARCELL CODE

User-Friendly marrow-cell kinetics calculations can be completed as described in Section 1. The development of the User-Friendly Input/Output interface routines was sponsored by The U.S. Department of Energy at Oak Ridge National Laboratory under contract DE-AC0584OR21400 with Martin Marietta Energy Systems, Inc. and The Great Lakes College Association. Sample computer screens that prompt user input for a hypothetical three component exposure are illustrated as follows in parts 2.2 to 2.11.

(Note: Bold type in the menu list indicates values that were entered by the user and underline type is used to indicate a selection chosen by the user.)

#### 2.1 Screen 1 of MarCell Code:

##### Main Menu

1. cell kinetics calculations.
2. cell kinetics calculations with 4 week recovery.
3. nuclear fallout calculations.
4. nuclear fallout calculations with 4 week recovery.
5. retrieve a set of calculations from disk.
6. view information about this program.
7. exit

Choose an option.

#### 2.2 Screen 2 of MarCell Code:

##### Number of Cell Types

1. 1 or more cell types of your choice.
2. 2 cell types: stem and stromal.

Choose an option.

#### 2.3 Screen 3 of MarCell Code:

##### Number of Cell Types

1. Mouse
2. Rat
3. Dog
4. Sheep
5. Swine
6. Burro
7. Man
8. Other (enter constants manually)

Choose an option

## 2.4 Screen 4 of MarCell Code:

### Radiation Types:

1. Tritium beta
2. 100 kVp x-rays
3. 250 kVp x-rays
4. Cobalt 60
5. Cesium 137
6. 2 MeV electrons
7. 22 MV x-rays
8. Neutron Fission
9. Neutron Fusion
10. Neutron:Gamma (1:1)
11. Neutron:Gamma (5:1)
12. Neutron:Gamma (30:1)
13. Other (enter rate constants manually)

Choose an option.

## 2.5 Screen 5 of MarCell Code:

### Dose Information

number of radiation doses (maximum of 500). > 3

marrow dose 1 (in cGy) > 100

marrow dose rate for dose 1 (in cGy/minute) > 100

time needed for dose 1: 1 minute(s), 0 second(s)

time (in minutes) between dose 1 and dose 2 > 1440

marrow dose 2 (in cGy) > 100

marrow dose rate for dose 2 (in cGy/minute) > 10

time needed for dose 2: 10 minutes, 0 seconds

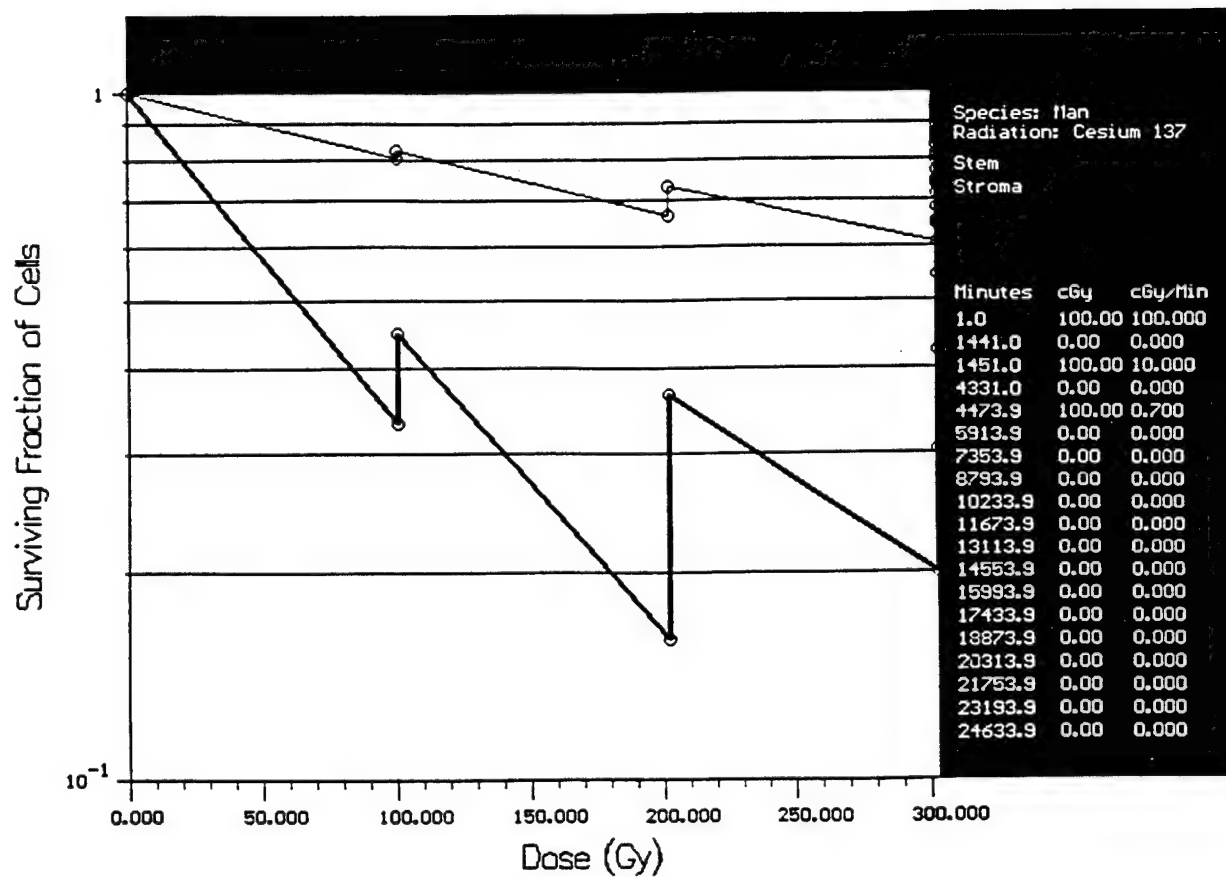
time (in minutes) between dose 2 and dose 3 > 2880

marrow dose 3 (in cGy) > 100

marrow dose rate for dose 3 (in cGy/minute) > 0.7

(press enter key)

## 2.6 Screen 6 of MarCell Code:



Press Any Key to Continue

Figure 2-1. Screen number 6 of the MarCell Code.

## 2.7 Screen 7 of MarCell Code:

### Treatment After Radiation Exposure

no treatment

antibiotics and blood cell prophylaxis

marrow transplant (radiation risk)

### Probability of Death

0.24

0.01

0.00

Press any key to continue.

## 2.8 Screen 8 of MarCell Code:

### Radiation Induced Risk

Cytopenia:		
Lifetime risk of all cancers		= 0.135
Lifetime risk of leukemia	= 0.019	
Repopulation:		
Lifetime risk of all cancers		= 0.226
Lifetime risk of leukemia	= 0.032	
DREF Factors:		
Cytopenia		= 0.633
Repopulation		= 1.060
Treatment Dose	= 300.000	
Effective Dose based on cytopenia	= 189.791	
Effective Dose based on repopulation	= 317.962	

(Note: Cellular repopulation during treatment may actually increase the cell killing effectiveness of a particular dose. Likewise, protracted irradiation may kill more cells than are actually present and when this occurs it is not possible to calculate an **equivalent prompt dose**. If this occurs, the EPDs are set to 999999.0000 and the corresponding risk estimates are meaningless.)

Equivalent Prompt Dose:	
Cytopenia	= 189.791
Repopulation	= 317.962

Press Any Key to Continue

## 2.9 Screen 9 of MarCell Code:

Cell Type	Equivalent Prompt Dose
Stem	140.287 (with respect to 250 kVp x-rays)
Stem	189.791 (with respect to cobalt 60)
Stroma	176.219 (with respect to 250 kVp x-rays)
Stroma	238.303 (with respect to cobalt 60)

Press any key to continue.

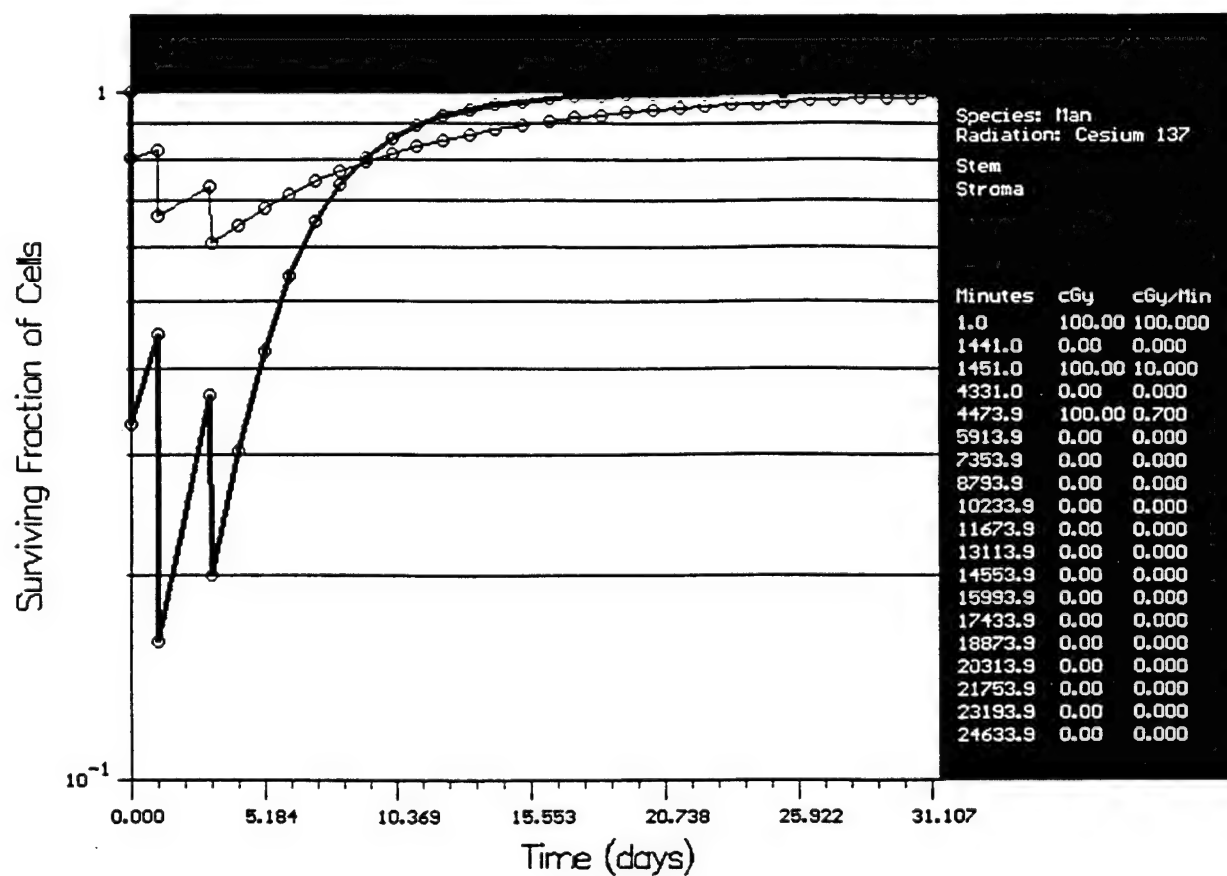
## 2.10 Screen 10 of MarCell Code:

### Options

1. Plot cell survival as a function of dose.
2. Plot cell survival as a function of time.
3. Save this set of calculations.
4. Return to the Main Menu

Choose an option.

## 2.11 Screen 11 of MarCell Code:



Press Any Key to Continue

Figure 2-2. Screen 11 of the MarCell Code.

The user can exit or loop to the next calculation.

### SECTION 3

#### DOSE-RATE RBE (OR DREF) FACTORS FOR PHOTONS

Traditionally, dose-response modeling has been on a strict experiment-by-experiment basis. Such an approach greatly restricts understanding of complex biological systems affected by numerous confounding factors that individually vary from experiment to experiment. In contrast, work described in this manuscript relies on a new analytical process (that considers both pooled and experiment-specific considerations) that was used to jointly analyze the bone marrow cell kinetics from a large data base on six species of test animals irradiated by protracted schedules of ionizing photon radiations. From this approach, we have modeled how the human  $LD_{50}$  may vary with dose protraction and how the dose rate efficiency or RBE factors for X rays,  $^{137}\text{Cs}$ , and  $^{60}\text{Co}$  change for irradiations given at constant rate over a minute, hour, day, week, and month.

An early achievement was to standardize animal dose-response data taken from diverse radiobiological experiments in which mortality (between 5 and 60 d post treatment) was measured under widely ranging conditions. Variables included dose, dose rate, number of dose fractions, species, and strain. Data used were taken from many investigators, several research laboratories, and treatment protocols comprised of prompt and protracted irradiations with various X and gamma photon sources. Most of the data used were published between 1960 and 1970.

After animal dose-response data were standardized for experimental design and species-strain related effects, experimental results were extrapolated to humans through simple scaling of a few remaining variables, such as the species-specific cycle time needed for cellular repopulation, the species-specific amount of DNA per diploid cell, and the linear energy transfer from the irradiating photons. The first and third considerations are quite important, but the DNA-per-cell correction was small and can be ignored for many applications.

From these analyses of data associated with protracted irradiations of test animals, two sets of dose-rate RBEs were computed. The RBEs were computed for cytopenia and toxicity-induced compensatory cell proliferation of stromal cells. In addition, other data were used to compute the corresponding RBEs for hematopoietic stem cells. Exposures considered were for X rays of 100 kVp and 250 kVp,  $^{137}\text{Cs}$ , and  $^{60}\text{Co}$  given at constant rates over a minute, hour, day, week, and month. The cytopenia based factors are useful

for considerations of immune system depression and acute mortality, whereas the cell proliferation based factors are useful as an index of cancer promotion (Jones et al. 1983; Jones 1984; NCRP 1989).

Since about 1990, there have been many biological experiments designed to better define the mechanistic relationship between compensatory cellular proliferation and cancer promotion. Historically, there have been several incorrect interpretations of biological experiments focused on this possible linkage. Most of these logical errors can be grouped into two categories: (1) compensatory cell proliferation was induced by a test compound without prior treatment with a small, subcarcinogenic, dose of a cancer initiating agent and (2) not recognizing the difference between initial toxic hyperplasia resulting from a single treatment and chronic irritation resulting from repeated treatments. Certain doses of many compounds cause initial hyperplasia which disappears with chronic exposure to the same concentration--a biological tolerance is acquired (Jones et al. 1983).

With respect to acute mortality (i.e., death within 60 d post-exposure), ionizing radiation may cause death by three different mechanisms or syndromes: injury to the hematopoietic bone marrow; the gastrointestinal tract; or the central nervous system. In man, large doses of ionizing radiations may reduce granulocyte and neutrophil counts in blood so that death from infection and/or hemorrhage can occur generally between about 9 and 45 d. Marrow doses in the range of 2 to 5 Gy (low LET photons given at high dose rates) typically span the graded response range from no mortality to no survival. At higher doses, i.e., 5 to 10 Gy, the gut begins to be the cause of death and individuals die (typically 2 to 5 d post exposure) before marrow damage becomes fully expressed. Above 10 Gy, death may occur within 24 h from damage to the central nervous system. If survival time and/or task performance is of interest, it is necessary to model the dose-response for all three syndromes. However, if risk of death is the consideration of interest, then it is adequate to simply model the dose-response of the hematopoietic marrow. A dose-response model for human mortality can be established as follows:

The first step is to use the empirically common condition that the  $LD_{90}/LD_{10} = 2$ . The second step involves assumed symmetry for the low-dose and high-dose tails of the probability distribution function. Taken together these two conditions provide simple algebraic estimates of  $LD_{90} = (4/3) \bullet LD_{50}$  and  $LD_{10} = (2/3) \bullet LD_{50}$ .



Values in column 2 of Table 3-1 were computed based on the assumption that man, irradiated outside of standard clinical conditions, could resemble a new, untested, biological species of 70 kg bodyweight drawn at random from the same probability distribution of radiosensitivities that provided the 13 species of test animals used in the mathematical analysis (Morris et al. 1989). We concluded that man irradiated and left to subsist under chaotic conditions (e.g., the initial Japanese Survivors in Hiroshima and Nagasaki) may--by environmental happenstance--be far more radiosensitive than man treated under standard clinical conditions.

Evaluations of Japanese data by investigators at Hiroshima University (Hayakawa et al. 1986) and the Radiation Effects Research Foundation (Fujita et al. 1989) have provided independent  $LD_{50}$  estimates only 20% higher than our estimate that was derived exclusively from test animals studied under standard "caged" conditions.

These estimates for the human  $LD_{50}$  are in close agreement with the discussion given by (Bond and Robertson 1957) based on (1) large animal radiobiology, (2) Marshallese exposed to early fallout, and (3) patients receiving whole-body irradiation.

More recently, (Levin et al. 1992) identified a population of young adults who were inside reinforced concrete buildings that remained structurally protective of blast and thermal injuries after the bombing of Nagasaki. Levin et al. computed the  $LD_{50}$  to be 2.90 Gy to marrow based on 75 individuals. This estimate serves as our normalizing point for the "normal"  $LD_{50}$  values in Table 3-1. The slope of the dose-response curve could not be accurately or realistically estimated from the data used in the Levin analysis, so we used the  $LD_{90} = (4/3) \bullet LD_{50}$  and  $LD_{10} = (2/3) \bullet LD_{50}$  assumptions to calculate a probit slope of  $1/0.744 \text{ Gy}^{-1}$  (i.e., the inverse of the standard deviation) for the median lethal dose of 2.90 Gy.

Fliedner et al. (1984) noted that the  $LD_{50}$  may be increased by a factor of 3 or 4 when new marrow is engrafted or by 1.5 or 2 when antibiotics and blood replacement therapy is used. These values seem somewhat greater than usually observed from experimental treatments but may represent ideal conditions including perfect clinical care. Therefore, we used arbitrary factors of 4 and 2, respectively, to demonstrate how our human "normal" estimates could be extended to the "transfusion" and "therapy" considerations [our methods can be used with different data, assumptions, or even for factors associated with cytokine therapy such as granulocyte-macrophage colony-stimulating factor (GM-CSF)].

The analysis described in this paper depends upon coupling two models both of which depends upon large data bases. The data base used to develop the mortality model was comprised of constant dose rate LD<sub>50</sub> experiments, and the data base used to develop the cell kinetics model was comprised of animal mortality data from complex protracted irradiations. The model for mortality has been documented previously (Jones 1981; Morris et al. 1989, 1988), as has the second model for bone marrow cellular kinetics (Jones et al. 1991, 1993a, 1993b; Morris et al. 1991, 1993).

From our completed calculations for mice, rats, dogs, sheep, swine, and burros, we have estimated cellular rate constants for marrow stroma and CFY-S cells (i.e., hematopoietic stem cells) as described in SECTION 1. The RBEs for many doses given at 0.50, 0.20, 0.10, 0.05, 0.02, and 0.01 Gy min<sup>-1</sup> are given in Table 3-2 for stromal tissues and Table 3-3 for hematopoietic stem cells.

For example, if a person was accidentally exposed to 0.01 Gy of <sup>137</sup>Cs over a month, the effective dose for stromal cell cytopenia would be  $(0.32) \times (0.01 \text{ Gy}) = 0.0032 \text{ Gy}$  of <sup>60</sup>Co. In contrast, the effective dose for stromal cell compensatory cell proliferation would be  $(1.2) \times (0.01 \text{ Gy}) = 0.012 \text{ Gy}$  of <sup>60</sup>Co, exactly the same as if the 0.01 Gy of <sup>137</sup>Cs was given in 1 min.

At higher dose, the non-linear and dose-rate dependent effects become more significant. Assume, for example, that a therapeutic fraction of 3 Gy was given over a period of 1 hr. This dose rate is representative of a protocol actually used to avoid emesis in marrow transplant candidates.

Table 3-1. Estimates of the human LD<sub>50</sub> and percentage survival for stromal and hematopoietic stem cells (HSC) for "Caged," "Normal," "Therapeutic," and "Transfusion" personal treatment conditions at dose rates ranging from 0.01 to 0.50 Gy min<sup>-1</sup>. "Caged" estimates are values for man that result from extrapolations of data from test animals, and "Normal" are based on the human LD<sub>50</sub> as determined by (Levin et al. 1992). The values for "Therapy" are based on Levin's estimate and on an assumed availability of antibiotics, irradiated blood elements, and perhaps therapeutic cytokines, whereas the values for "Transfusion" also include marrow infusion for engraftment--see the text.

Dose Rate (Gy min <sup>-1</sup> )	"Cage" LD <sub>50</sub> (Gy)	Stroma (percent)(percent)	HSC (percent)(percent)	"Normal" LD <sub>50</sub> (Gy)	Stroma (percent)(percent)	HSC (percent)(percent)	"Therapy" LD <sub>50</sub> (Gy)	Stroma (percent)(percent)	HSC (percent)(percent)	"Transfusion" LD <sub>50</sub> (Gy)	Stroma (percent)(percent)	HSC (percent)(percent)
0.50	1.83	53	15	2.90	34	4.9	5.80	8.9	0.22	11.60	0.51	0.00043
0.20	2.06	49	13	3.25	30	3.7	6.50	7.1	0.13	13.00	0.38	0.00015
0.10	2.24	46	11	3.54	27	3.2	7.08	6.4	0.098	14.16	0.36	0.000093
0.05	2.45	44	11	3.87	26	3.0	7.74	6.1	0.094	15.48	0.37	0.000090
0.02	2.74	42	12	4.33	25	3.7	8.66	6.2	0.15	17.32	0.40	0.00026
0.01	2.99	41	15	4.72	25	5.7	9.44	6.6	0.40	18.88	0.49	0.0022

Table 3-2.

Dose-Rate dependent RBEs were computed based on how different irradiation patterns affect the nadir of the human stroma-cell survival curve (curve is a function of time for a particular dose schedule).

Dose (Gy)	EXPOSURE PERIOD				
	1 min	1 h	1 d	1 wk	1 mo
<b>I. <sup>60</sup>Co photons at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.0 (1.0)	1.0 (1.0)	0.94 (1.0)	0.68 (1.0)	0.27 (1.0)
0.05	1.0 (1.0)	1.0 (1.0)	0.94 (0.99)	0.68 (0.99)	0.27 (0.99)
0.10	1.0 (1.0)	1.0 (1.0)	0.92 (0.98)	0.67 (0.98)	0.27 (0.98)
0.25	1.0 (1.0)	1.0 (0.98)	0.91 (0.95)	0.64 (0.95)	0.27 (0.95)
0.50	1.0 (1.0)	0.98 (0.97)	0.86 (0.90)	0.62 (0.90)	0.26 (0.91)
1.00	1.0 (1.0)	0.95 (0.95)	0.81 (0.84)	0.59 (0.84)	0.25 (0.86)
1.50	1.0 (1.0)	0.94 (0.93)	0.77 (0.80)	0.56 (0.80)	0.24 (0.83)
2.00	1.0 (1.0)	0.93 (0.93)	0.73 (0.78)	0.54 (0.78)	0.23 (0.82)
2.50	1.0 (1.0)	0.92 (0.93)	0.72 (0.77)	0.52 (0.77)	0.23 (0.82)
3.00	1.0 (1.0)	0.91 (0.92)	0.70 (0.76)	0.50 (0.77)	0.22 (0.83)
3.50	1.0 (1.0)	0.91 (0.92)	0.68 (0.76)	0.49 (0.77)	0.22 (0.84)
4.00	1.0 (1.0)	0.90 (0.93)	0.67 (0.76)	0.48 (0.78)	0.22 (0.85)
4.50	1.0 (1.0)	0.90 (0.93)	0.66 (0.76)	0.47 (0.78)	0.21 (0.87)
<b>II. <sup>137</sup>Cs photons at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.2 (1.2)	1.2 (1.2)	1.1 (1.2)	0.80 (1.2)	0.32 (1.2)
0.05	1.2 (1.2)	1.2 (1.2)	1.1 (1.2)	0.80 (1.2)	0.32 (1.0)
0.10	1.2 (1.2)	1.2 (1.2)	1.1 (1.2)	0.79 (1.2)	0.32 (1.0)
0.25	1.2 (1.2)	1.2 (1.2)	1.1 (1.1)	0.76 (1.1)	0.32 (0.99)
0.50	1.2 (1.2)	1.1 (1.2)	1.0 (1.1)	0.74 (1.1)	0.31 (0.98)
1.00	1.2 (1.2)	1.1 (1.1)	0.94 (0.98)	0.68 (0.99)	0.29 (0.98)
1.50	1.2 (1.2)	1.1 (1.1)	0.89 (0.94)	0.65 (0.94)	0.29 (0.96)
2.00	1.2 (1.2)	1.1 (1.1)	0.86 (0.90)	0.62 (0.91)	0.28 (0.94)
2.50	1.2 (1.2)	1.1 (1.1)	0.83 (0.89)	0.60 (0.90)	0.27 (0.93)
3.00	1.2 (1.2)	1.1 (1.1)	0.81 (0.87)	0.58 (0.89)	0.26 (0.92)
3.50	1.2 (1.1)	1.1 (1.1)	0.79 (0.87)	0.57 (0.89)	0.25 (0.91)
4.00	1.2 (1.1)	1.1 (1.0)	0.78 (0.86)	0.55 (0.89)	0.25 (0.89)
4.50	1.2 (1.1)	1.1 (1.0)	0.76 (0.86)	0.54 (0.89)	0.24 (0.88)
<b>III. 250 kVp X rays at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.4 (1.4)	1.3 (1.4)	1.3 (1.4)	0.91 (1.4)	0.37 (1.4)
0.05	1.4 (1.4)	1.3 (1.4)	1.3 (1.3)	0.90 (1.3)	0.36 (1.3)
0.10	1.4 (1.4)	1.3 (1.4)	1.2 (1.3)	0.89 (1.3)	0.36 (1.3)
0.25	1.4 (1.4)	1.3 (1.3)	1.2 (1.3)	0.88 (1.3)	0.36 (1.3)
0.50	1.4 (1.4)	1.3 (1.3)	1.1 (1.2)	0.82 (1.2)	0.35 (1.2)
1.00	1.4 (1.4)	1.3 (1.3)	1.1 (1.1)	0.76 (1.1)	0.33 (1.1)
1.50	1.4 (1.4)	1.3 (1.3)	1.0 (1.1)	0.72 (1.1)	0.32 (1.1)
2.00	1.4 (1.3)	1.2 (1.2)	0.96 (1.0)	0.69 (1.0)	0.31 (1.0)
2.50	1.4 (1.3)	1.2 (1.2)	0.92 (0.99)	0.67 (1.0)	0.30 (1.0)
3.00	1.4 (1.3)	1.2 (1.2)	0.90 (0.97)	0.65 (0.99)	0.29 (0.99)
3.50	1.4 (1.2)	1.2 (1.2)	0.88 (0.95)	0.63 (0.98)	0.28 (0.98)
4.00	1.4 (1.2)	1.2 (1.1)	0.87 (0.94)	0.62 (0.98)	0.28 (0.98)
4.50	1.4 (1.2)	1.2 (1.1)	0.85 (0.94)	0.60 (0.98)	0.27 (0.98)
<b>IV. 100 kVp X rays at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.6 (1.5)	1.5 (1.5)	1.4 (1.5)	1.0 (1.5)	0.42 (1.5)
0.05	1.6 (1.5)	1.5 (1.5)	1.4 (1.5)	1.0 (1.5)	0.42 (1.5)
0.10	1.6 (1.6)	1.5 (1.5)	1.4 (1.5)	1.0 (1.5)	0.41 (1.5)
0.25	1.6 (1.6)	1.5 (1.5)	1.4 (1.4)	0.98 (1.4)	0.40 (1.5)
0.50	1.6 (1.6)	1.5 (1.5)	1.3 (1.4)	0.92 (1.4)	0.39 (1.4)
1.00	1.6 (1.6)	1.4 (1.5)	1.2 (1.3)	0.86 (1.3)	0.37 (1.3)
1.50	1.6 (1.5)	1.4 (1.4)	1.1 (1.2)	0.81 (1.2)	0.36 (1.3)
2.00	1.6 (1.5)	1.4 (1.4)	1.1 (1.1)	0.77 (1.1)	0.35 (1.2)
2.50	1.6 (1.4)	1.4 (1.3)	1.0 (1.1)	0.74 (1.1)	0.33 (1.2)
3.00	1.6 (1.4)	1.4 (1.3)	1.0 (1.1)	0.72 (1.1)	0.32 (1.2)
3.50	1.6 (1.3)	1.4 (1.3)	0.99 (1.0)	0.70 (1.1)	0.32 (1.2)
4.00	1.6 (1.3)	1.4 (1.2)	0.97 (1.0)	0.69 (1.1)	0.31 (1.2)
4.50	1.6 (1.3)	1.4 (1.2)	0.96 (1.0)	0.67 (1.1)	0.30 (1.2)

**Table 3-3.** Dose-Rate RBEs were computed for hematopoietic stem cells. Values outside parentheses were computed based on how different irradiation patterns affect the nadir of the human hematopoietic stem-cell curve (curve is a function of time for a particular treatment schedule).

Dose (Gy)	EXPOSURE PERIOD				
	1 min	1 h	1 d	1 wk	1 mo
<b>I. <sup>60</sup>Co photons at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.0 (1.0)	0.94 (0.94)	0.79 (0.91)	0.39 (0.91)	0.10 (0.90)
0.05	1.0 (1.0)	0.85 (0.82)	0.65 (0.69)	0.34 (0.69)	0.10 (0.69)
0.10	1.0 (1.0)	0.80 (0.76)	0.57 (0.57)	0.31 (0.56)	0.095 (0.56)
0.25	1.0 (1.0)	0.76 (0.72)	0.47 (0.44)	0.25 (0.43)	0.086 (0.43)
0.50	1.0 (1.0)	0.75 (0.76)	0.40 (0.42)	0.21 (0.39)	0.077 (0.40)
1.00	1.0 (1.0)	0.78 (0.84)	0.36 (0.47)	0.18 (0.43)	0.065 (0.44)
1.50	1.0 (1.0)	0.81 (0.89)	0.35 (0.55)	0.16 (0.50)	0.059 (0.52)
2.00	1.0 (1.0)	0.82 (0.93)	0.35 (0.62)	0.15 (0.58)	0.055 (0.61)
2.50	1.0 (1.0)	0.84 (0.95)	0.35 (0.70)	0.14 (0.66)	0.052 (0.71)
3.00	1.0 (1.0)	0.86 (0.97)	0.35 (0.76)	0.14 (0.74)	0.049 (0.81)
3.50	1.0 (1.0)	0.87 (0.98)	0.36 (0.82)	0.14 (0.82)	0.047 (0.92)
4.00	1.0 (1.0)	0.88 (0.99)	0.37 (0.87)	0.14 (0.90)	0.045 (1.0)
4.50	1.0 (1.0)	0.89 (0.99)	0.38 (0.91)	0.13 (0.98)	0.044 (1.1)
<b>II. <sup>137</sup>Cs photons at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.2 (1.2)	1.1 (1.1)	0.90 (1.0)	0.44 (1.0)	0.12 (1.0)
0.05	1.2 (1.2)	0.97 (0.98)	0.73 (0.80)	0.39 (0.79)	0.11 (0.79)
0.10	1.2 (1.2)	0.93 (0.92)	0.64 (0.65)	0.34 (0.64)	0.11 (0.64)
0.25	1.2 (1.2)	0.89 (0.88)	0.52 (0.51)	0.28 (0.49)	0.097 (0.49)
0.50	1.2 (1.2)	0.89 (0.91)	0.45 (0.49)	0.24 (0.45)	0.085 (0.46)
1.00	1.2 (1.1)	0.93 (0.96)	0.42 (0.54)	0.20 (0.49)	0.073 (0.51)
1.50	1.2 (1.1)	0.97 (0.99)	0.41 (0.62)	0.18 (0.57)	0.066 (0.60)
2.00	1.2 (1.1)	1.0 (1.0)	0.41 (0.71)	0.17 (0.66)	0.061 (0.70)
2.50	1.2 (1.0)	1.0 (1.0)	0.42 (0.78)	0.16 (0.75)	0.057 (0.81)
3.00	1.2 (1.0)	1.0 (1.0)	0.43 (0.84)	0.16 (0.78)	0.055 (0.92)
3.50	1.2 (1.0)	1.0 (1.0)	0.44 (0.89)	0.16 (0.92)	0.052 (1.0)
4.00	1.2 (1.0)	1.1 (1.0)	0.45 (0.94)	0.15 (1.0)	0.051 (1.2)
4.50	1.2 (1.0)	1.1 (1.0)	0.46 (0.97)	0.15 (1.1)	0.049 (1.3)
<b>III. 250 kVp X rays at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.4 (1.4)	1.2 (1.3)	1.0 (1.2)	0.51 (1.2)	0.14 (1.2)
0.05	1.4 (1.5)	1.1 (1.2)	0.83 (0.93)	0.44 (0.92)	0.13 (0.92)
0.10	1.4 (1.5)	1.1 (0.92)	0.72 (0.76)	0.39 (0.74)	0.12 (0.74)
0.25	1.4 (1.4)	1.0 (1.0)	0.58 (0.60)	0.32 (0.60)	0.11 (0.56)
0.50	1.4 (1.3)	1.0 (1.0)	0.51 (0.56)	0.25 (0.52)	0.096 (0.53)
1.00	1.4 (1.2)	1.1 (1.0)	0.47 (0.62)	0.22 (0.57)	0.081 (0.59)
1.50	1.4 (1.1)	1.1 (1.0)	0.47 (0.71)	0.20 (0.65)	0.073 (0.69)
2.00	1.4 (1.1)	1.1 (1.0)	0.48 (0.78)	0.19 (0.75)	0.068 (0.80)
2.50	1.4 (1.1)	1.2 (1.0)	0.49 (0.85)	0.18 (0.84)	0.064 (0.93)
3.00	1.4 (1.0)	1.2 (1.0)	0.50 (0.91)	0.18 (0.94)	0.061 (1.1)
3.50	1.4 (1.0)	1.2 (1.0)	0.52 (0.95)	0.18 (1.0)	0.058 (1.2)
4.00	1.4 (1.0)	1.2 (1.0)	0.53 (0.98)	0.18 (1.1)	0.057 (1.3)
4.50	1.4 (1.0)	1.2 (1.0)	0.55 (1.0)	0.18 (1.2)	0.055 (1.5)
<b>IV. 100 kVp X rays at different doses and dose rates compared with pulse irradiation by <sup>60</sup>Co.</b>					
0.01	1.6 (1.6)	1.4 (1.5)	1.2 (1.4)	0.57 (1.4)	0.16 (1.4)
0.05	1.6 (1.8)	1.3 (1.4)	0.92 (1.1)	0.49 (1.0)	0.15 (1.0)
0.10	1.6 (1.8)	1.2 (1.3)	0.80 (0.87)	0.43 (0.85)	0.14 (0.85)
0.25	1.6 (1.7)	1.2 (1.2)	0.65 (0.69)	0.35 (0.65)	0.12 (0.65)
0.50	1.6 (1.5)	1.2 (1.2)	0.58 (0.65)	0.29 (0.59)	0.11 (0.71)
1.00	1.6 (1.3)	1.3 (1.2)	0.54 (0.71)	0.25 (0.64)	0.090 (0.67)
1.50	1.6 (1.2)	1.3 (1.1)	0.44 (0.79)	0.22 (0.74)	0.081 (0.78)
2.00	1.6 (1.1)	1.4 (1.1)	0.56 (0.87)	0.21 (0.84)	0.074 (0.91)
2.50	1.6 (1.1)	1.4 (1.1)	0.58 (0.93)	0.21 (0.94)	0.070 (1.1)
3.00	1.6 (1.0)	1.4 (1.0)	0.60 (0.97)	0.20 (1.0)	0.067 (1.2)
3.50	1.6 (1.0)	1.4 (1.0)	0.62 (1.0)	0.20 (1.1)	0.064 (1.4)
4.00	1.6 (1.0)	1.4 (1.0)	0.64 (1.0)	0.20 (1.2)	0.062 (1.5)
4.50	1.6 (1.0)	1.4 (1.0)	0.66 (1.0)	0.20 (1.3)	0.061 (1.6)

## SECTION 4

### DOSE-RATE RBE FACTORS FOR NEUTRONS

#### 4.1 SUMMARY.

The objectives of this study were to: (a) extend previous bone-marrow cell kinetics models that have been published for ionizing photons to include neutron radiations, and (b) provide Relative Biological Effectiveness (RBE) values for time-specific cell killing (cytopenia) and compensatory cellular proliferation (repopulation in response to toxic injury) for neutron doses ranging from 0.01 to 4.5 Gy delivered uniformly over a minute, hour, day, week, and month. The RBEs presented are for "one radiation compared with another is the inverse ratio of the absorbed doses producing the same degree of a defined biological endpoint" as worded by the ICRP. RBEs for cytopenia of a cell lineage were based on ratios of protocol-specific doses that determined the same cell population nadir; whereas, the RBEs for repopulation of a lineage were based on the ratios of protocol-specific doses that determined the same total number of cells killed over the radiation treatments, and which should be replaced for long-term survival of the animal. Time-dependent RBEs were computed for neutron exposures relative to the effect of  $^{60}\text{Co}$  given as a prompt dose. By the use of these RBE factors, low or variable dose rates, dose fractionations given over long periods of time, and different protocols involving several radiation qualities can be converted realistically, and by standard convention, into an *equivalent dose* of a reference radiation comprised of X or  $\gamma$  rays given either as a pulse or at any other reference dose rate for which risk information based on epidemiological or animal dose-response data are available. For stromal tissues irradiated by fission neutrons, time-dependent RBEs for cytopenia were computed to range from 4.24 to 0.70 and RBEs for repopulation varied from a high of 6.88 to a low of 2.24. For hematopoietic stem cells irradiated by fission neutrons, time-dependent RBEs for cytopenia were computed to range from 5.02 to 0.22 and RBEs for repopulation varied from a high of 5.02 to a low of 1.98. RBEs based on tissue-kerma-free-in-air would be about two fold higher for isotropic cloud or rotational exposure geometries. For certain doses and dose rates, the RBE values computed for compensatory cellular proliferation clearly demonstrate the behavior that is commonly referred to as an inverse dose-rate effect, i.e., protraction of exposure may--under certain conditions--increase the magnitude of the dose response. Furthermore, because of non-linear rates for repair and repopulation, the highest RBEs are not necessarily found for the lowest doses nor the lowest RBEs always found at the highest doses.

## 4.2 INTRODUCTION.

During a symposium at the 30th anniversary of the Armed Forces Radiobiology Research Institute, (Ainsworth 1991) and (Alpen 1991) provided historical reviews of neutron radiobiology. Some additional perspective can be gained from NCRP Report 104 on "The Relative Biological Effectiveness of Radiations of Different Quality" (NCRP 1990). Our present analysis of neutron-induced marrow-cell kinetics depends substantially on experiments conducted in the research climate described in these three references. Without the comprehensive array of in vivo and in vitro experiments from the 1950's and 1960's, our modeling studies would not be possible according to either the logic or the type of data that we have used.

Neutron calculations also depend upon previous models for compensatory cell kinetics that are associated with animal mortality resulting from X- and  $\gamma$ -irradiations. The logic and data used for those photon models also identify largely with that same research period. The composite model for cells that are "critical" to hematopoiesis [published for ionizing photons (Jones et al. 1993a and 1993b; Morris et al. 1993) and to be developed here for neutron radiations] is comprised of two constituent models: (a) a **cellular model** and (b) a **mortality model**.

The mortality component of the model depends upon a normal probability (probit) density function specified by an  $LD_{50}$  and standard deviation  $\sigma$  for each experiment of interest. The choice of the probit function has been described by (Morris et al. 1988 and 1989). Our estimates of rate constants for the compensatory cell kinetics are derived from acute hematopoietic mortality caused by ionizing photons (Jones et al. 1991, 1993a, and 1993b; Morris et al. 1991 and 1993). Cellular processes were described by compartmental modeling according to transitions of cells between normal, injured, and killed states. For ionizing photons, considerations included sublethal injury, repair of sublethal injury, 1-hit killing, 2-hit killing, and compensatory repopulation.

## 4.3 MATERIALS AND METHODS.

**Model:** Our published photon cell-kinetics models contain terms that can accommodate the high-LET component of low-LET photon radiations; however, from most neutron irradiations of animals or cells there seems to be little, if any, repair of sublethal cellular injury so a simplified model is prudent [e.g. see NCRP Report 104 (NCRP 1990)]. However, from physical processes involved, it seems certain that some reaction products would result in sublethal injury to some cells, but, overall, it appears that the injured population of cells that can be repaired is simply too small to observe against

a high background of cells killed by 1-hit kinetics. From a wealth of such experimental data, we (for purposes of evaluating coefficients of the model) have assumed that the compensatory cell kinetics associated with neutron irradiations need not include considerations for sublethal injury. Without sublethal injury there is no need for modeling the repair of sublethal injury and killing of sublethally injured cells, i. e., 2-hit killing. Thus, the cell-kinetics model used for neutron irradiations was reduced from the 5-coefficient photon model to simple 1-hit killing with a rate coefficient of  $\lambda_{NK}$  ( $\text{Gy}^{-1}$ ), plus repopulation with a rate coefficient of  $\lambda_{NN}$  ( $\text{min}^{-1}$ ). This simplification results in the two coefficient cellular model illustrated in the upper left panel of Figure 4-1. The rate that cells are killed is dependent on the product of  $\lambda_{NK}$  ( $\text{Gy}^{-1}$ ) and dose rate ( $\text{Gy min}^{-1}$ ); whereas, the rate of repopulation is dependent on  $\lambda_{NN}$  ( $\text{min}^{-1}$ ). The neutron model is, therefore, a simple, special case of the photon model with **only one new coefficient** to be estimated from neutron acute-mortality data. With this one additional coefficient ( $\lambda_{n,k}$ )<sub>neutron</sub>, protracted irradiations with photon radiations can be equated with its same intra-experimental neutron counterpart. [As will be seen later in this paper, the abbreviated neutron model, having only one coefficient of its own, seems to be no less accurate than the full 5-coefficient model that was used for ionizing photon radiations.]

#### 4.3.1 Mortality Data and Stromal Cells.

Mortality data from protracted irradiations were used to estimate model coefficients for X- and  $\gamma$ -ray exposures. Although the neutron model contains two cellular rate constants, only one is neutron specific (in contrast to five for the photon model), there are few animal-mortality data that we can use to independently model the cell kinetics that underlie hematopoietic death associated with neutron exposures--most such data are for simple continuous dose rates, typically above  $0.05 \text{ Gy min}^{-1}$ . Thus, treatment protocols are too brief to be useful for estimation of rate constants for compensatory repopulation, i.e., the typical repopulation doubling time is long relative to the course of the experimental irradiation. Very few experiments have been attempted where neutron dose rates were low, varied, fractionated, or otherwise protracted within a fixed experimental design.

The data from neutron mortality experiments summarized in Table 4-1 were used to calculate maximum likelihood estimates of  $\lambda_{NK}$  in the model as described for X and  $\gamma$  rays (Morris et al. 1991 and 1993)--the one additional constant in the model is  $\lambda_{NN}$  the rate constant for repopulation and for which a previous estimate based on X and  $\gamma$  ray data was used. This usage implicitly assumes that the same "critical" cellular lineage is depressed by both high and low LET radiations. From an exhaustive literature review, we have been unable to find any published experimental evidence that is inconsistent with this assumption. Both high- and low-LET radiations depress cell counts associated



with all lineages. Furthermore, comparisons that will be shown later in this paper indicate that there seems to be no reason to assume that another, different "critical" lineage is associated with irradiation by neutrons.

Repopulation in the model is simply time-dependent upon tissue necrosis, or need to heal at a particular instant. Thus, there is a simple implied dependence on tissue damage from dose, radiation quality, and dose rate all taken in combination. Therefore, the rate constant for compensatory proliferation was taken as the species-specific value computed from the data base on protracted photon irradiations (Morris et al. 1993). (This behavior will also be used for the neutron hematopoietic stem cell model to be described later in this section.)

Although, the neutron mortality experiments suffer from lack of protraction (i.e., they do not permit analysis of the rate of compensatory proliferation), they have one great advantage in that each neutron protocol was run in parallel with an X- or  $\gamma$ -ray counterpart. The unique flexibility of the mathematics determines a theoretical cell-survival curve for the "critical" lineage, and the particular level of cell survival from a course of treatment permits a protocol dose [at any rate(s) and/or number of fractionations] of neutron, X or gamma radiation to be converted into its equivalent prompt dose of a standard or reference radiation. In addition the coefficients of the prompt-dose mortality model, [i.e., the  $LD_{50}$  (Gy) and slope ( $Gy^{-1}$ ) which is the inverse of the standard deviation  $\sigma$  (Gy)] can be estimated from the matched photon experiment(s). Thus, for each neutron experiment used, we estimated only one coefficient (for the 1-hit killing by neutrons) by maximizing the likelihood function for the cellular model (from the neutron mortality data) and an  $LD_{50}$  and  $\sigma$  (both for the mortality model) from the joint neutron and matched X and/or  $\gamma$  companion mortality experiment. All determinations were made simultaneously for each neutron-photon set of data.

With simultaneous analysis of data representing different types of radiation, a practical reference radiation type or source must be selected upon which to base the equivalent prompt doses for different protocols with different radiation qualities. In the first evaluation paper by (Morris et al. 1991) on rate constants for mice, all treatments were from nominal 250 kVp X rays and the EPD was calculated accordingly. Different photon qualities were considered for the multi-species evaluation based on mice, rats, dogs, sheep, swine, and burros (Morris et al. 1993); this is also the situation for the X and/or  $\gamma$  companion experiments used here. For the current analysis, calculations were repeated using 100 kVp X rays, 250 kVp X rays, and  $^{60}Co$   $\gamma$  rays as the basis for the equivalent prompt dose, but the specific choice of a particular reference radiation for the cell survival trajectory

did not produce variations of practical importance in the parameter estimates for a particular radiation quality--of course, curves for cell-survival did change with the choice of the reference radiation. We chose  $^{60}\text{Co}$  to be consistent with our previous RBE dose-rate factors for X rays and gamma rays, and neutron coefficients are indexed to the cell survival determined by the photon coefficients described by (Morris et al. 1993).

In the study based on mice irradiated with nominal 250 kVp X-rays, Morris et al. (1991) found that any curve of a radio-resistance equal to or greater than the curve described as "a" would maximize the likelihood function. Later, an additional analysis was based on the mouse data used in the 1991 paper but supplemented with additional mouse data in addition to data for rats, dogs, sheep, swine, and burros. Also, nominal 120 kVp X-rays,  $^{60}\text{Co}$ , and  $^{137}\text{Cs}$  were included with the nominal 250 kVp data (Morris et al. 1993). Because of the additional diversity of the larger data base with many experiments having greater protraction of dose, the lower bound of the family of curves which could maximize the likelihood function was more precisely defined. The 1993 analysis produced a more radio-resistant lower bound. Although the bounding curve for 1993 differed significantly from that of 1991, both compare well with a stromal-like cell and are not characteristic of a stem-like cell. Because the 1993 data base included (a) more data on mice, (b) data on 5 other species, and (c) other photon radiations, the range of uncertainty of the lower bound could be reduced and resulted in a greater radioresistance. The evaluations, herein, are normalized to the 1993 photon coefficients taken to be the "gold" standard because of the diversity of data used in the optimization process.

Comparisons of modeled mortality vs observed mortality for the 7 studies are shown in Figure 4-2. The corresponding  $\lambda_{\text{NK}}$  estimates are given in column 7 of Table 4-2. Only point values are given for those estimates. Those estimates were derived under the assumption that our previous estimates of coefficients in the cell kinetics model for photons are exactly correct, rather than subject to random uncertainty or experimental bias. This may be reasonable for establishing point estimates of  $\lambda_{\text{NK}}$  but does not lead to realistic confidence intervals.

#### 4.3.2 Analysis of In Vitro Data.

In addition to model evaluations from neutron mortality data, published cell-survival curves for marrow stromal and fibroblast cells can also be used to estimate the  $\lambda_{\text{NK}}$  coefficient. From these cell-survival studies, the associated  $\lambda_{\text{NK}}$  values given in column 7 of Table 4-2 were made by evaluating S from

$$S = 1 - [ 1 - \exp (- D / D_0 ) ]^n$$

at 3 Gy and then solving the simple, prompt-dose, one-coefficient survival model for  $\lambda_{NK}$  ( $\text{Gy}^{-1}$ ) so that our non- $(D_0, n)$  cell-survival model passed through the same point at 3 Gy. Three Gy was used because some studies did not include higher doses. Results given in column 7, at the bottom of Table 4-2, seem consistent with estimates derived from maximum-likelihood analysis of animal mortality data.

#### 4.3.3 RBE'S for Stroma.

A previous study considered dose-rate dependent RBEs for cytopenia and compensatory cell proliferation resulting from exposure to ionizing photons (Jones et al. 1994a). RBE values for cytopenia and repopulation were described as functions of dose and dose rate for 100 kVp X rays, 250 kVp X rays,  $^{137}\text{Cs}$ , and  $^{60}\text{Co}$  given uniformly over a minute, hour, day, week, or month compared with prompt dose irradiation by  $^{60}\text{Co}$ . RBE values for doses given over long periods of time, even years, differ insignificantly from the values computed for a month.

The reference radiation for the neutron RBEs is prompt dose irradiation by  $^{60}\text{Co}$  as was used previously (Jones et al. 1994a; Morris et al. 1994c). The RBEs vary according to a dose and dose-rate surface described in the section on photon RBEs. Although each combination of radiation source, cell lineage, and cellular effect would produce a 3-dimensional surface, tabular data will be given because of its greater utility. RBE values for stroma, given in Table 4-3 for cytopenia and repopulation, were based on the  $\lambda_{n,k}$  values as follows:

#### 4.3.4 Stroma: Fission Neutrons.

Rodent studies by (Stewart et al. 1982), (Upton et al. 1956), (Delihas and Curtis 1958), and cell-survival studies by (Meijne et al. 1992) (3 experiments) determined a mean  $\lambda_{NK}$  of  $0.672 \text{ Gy}^{-1}$  with an associated standard deviation of  $0.037 \text{ Gy}^{-1}$ . We have used data which we have interpreted as showing that humans may have about 8% less DNA per cell than mice (Morris et al. 1993). Making an adjustment for DNA content per cell gives a value of about  $0.622 \text{ Gy}^{-1}$  that may be used to compute RBE values as given in Table 4-3.

#### 4.3.5 Stroma: Fusion Neutrons.

For humans, a composite  $\lambda_{NK}$  of  $0.458 \text{ Gy}^{-1}$  was used to compute RBE values as given in Table 4-3. The prompt-dose cell-survival curves for ionizing photons (Morris et al. 1993) and fission- and fusion-energy neutrons are shown in Figure 4-3.

#### **4.3.6 Hematopoietic Stem Cell Model.**

For hematopoietic stem cells, cell-survival and repopulation data in vivo can be used to evaluate the  $\lambda_{NK}$  and  $\lambda_{NN}$  coefficients of the model (Jones et al. 1993a). Those values for  $\lambda_{NK}$  are given in column 7 of Table 4-4.

#### **4.3.7 Stem Cells: Fission Neutrons.**

Studies by (Ainsworth et al. 1969), (Davids 1973), (Boyum et al. 1978), and (Carter et al. 1956) are consistent as shown in Table 4-4. The  $\lambda_{NK}$  of 2.2 Gy<sup>-1</sup> for mice irradiated by Ainsworth's use of a Triga reactor was adjusted to 2.0 Gy<sup>-1</sup> to correspond to human DNA.

#### **4.3.8 Stem Cells: Fusion Neutrons.**

Studies by (Hendry and Howard 1971), (Broerse et al. 1971), (Duncan et al. 1969), and (Ainsworth et al. 1969) are consistent with each other as seen in Table 4-4. For consistency between fission and fusion spectra, we chose to use Ainsworth's value of 1.4 Gy<sup>-1</sup> which gives 1.3 Gy<sup>-1</sup> when scaled to human DNA.

#### **4.3.9 RBE'S for Stem Cells.**

A previous study considered dose-rate dependent effects on stem cells for cytopenia and compensatory cell proliferation resulting from exposure to ionizing photons (Jones et al. 1993a). RBE values for cytopenia and repopulation were described as functions of dose and dose rate for 100 kVp X rays, 250 kVp X rays, <sup>137</sup>Cs, and <sup>60</sup>Co given uniformly over a minute, hour, day, week, or month compared with prompt-dose irradiation by <sup>60</sup>Co. Stem-cell RBE values for cytopenia and repopulation are given in Table 4-5. The prompt-dose cell-survival curves for ionizing photons and fission- and fusion-energy neutrons are shown in Figure 4-4.

#### **4.3.10 Example.**

Assume that a person is accidentally exposed to a fission neutron source at a daily marrow dose of 0.10 Gy for a week. The total exposure would be 0.70 Gy. the length of the daily exposure periods are mostly inconsequential for the neutron model. From Table 4-3 an RBE of about 2.13 (average of 2.26 and 2.01) for fission neutrons would be taken for cytopenia so that the effect should be equivalent to about 1.5 Gy of <sup>60</sup>Co given at a prompt rate. Without medical support such as antibiotics and irradiated blood elements, this might correspond to a probability of acute mortality of about 10%.

For risk of leukemia or other chronic hematological effects, an RBE for compensatory cell proliferation of about 3.19 (average of 3.31 and 3.08) could be used to estimate an effective prompt dose of about 2.2 Gy of  $^{60}\text{Co}$  which could be used with risk coefficients such as those promulgated by the U.S. National Academy of Sciences (in the BEIR Reports) or by the United Nations (in the UNSCEAR Reports).

#### 4.4 DISCUSSION.

Model coefficients for (a) cells critical to hematopoiesis, and (b) hematopoietic stem cells are summarized at the beginning of this report. The RBEs for cytopenia are useful to evaluate risk of acute effects such as radiation sickness and/or death and to develop protocols for immunosuppression, marrow ablation, and leukemia treatments, whereas the RBEs for compensatory cell proliferation should be useful for comparisons of cancer promotion and chronic risk (Jones et al. 1983; Jones 1984; NCRP 1989; IARC 1992).

The system of differential equations which comprise the cell-kinetics model is non-linear, and computed photon RBEs show a less-than-linear risk for X and  $\gamma$  rays. Although, the neutron models developed in this paper are based on simple 1-hit kinetics (no repair of sublethal injury—considered as a non-linear process for photons), a non-linear response (i.e., concave) can still be seen because of the factors associated with compensatory repopulation.

As summarized in Table 4-2, (Vogel et al. 1957) measured the biological effects of fission energy neutrons moderated through 9 cm of Pb. That moderation could shift the mean energy of the recoil proton spectrum closer to the Bragg peak of the stopping power curve. In this energy range, each neutron interaction has a high probability of producing a proton having about half of its own energy. Proton energies near the Bragg peak cause greater biological damage and that behavior seems consistent with the larger value of  $\lambda_{\text{NK}}$  shown for Vogel's experiment.

The Triga reactor used by (Stewart et al. 1982) was also Pb moderated (20 cm) but the  $\lambda_{\text{NK}}$  value which we estimated seems quite consistent with the other devices that produced a theoretically unmoderated or Watt-type fission spectrum. Stewart's experiment was shielded by Pb to produce a 30:1 neutron to gamma dose ratio and the effective energy shift for recoil protons may have been toward stopping powers significantly below the Bragg peak. Those lower energy protons could be far less toxic than protons near the Bragg peak or those resulting from the assembly used by Vogel.

#### 4.5 CONCLUSIONS.

NCRP in Report 104 used a linear-quadratic model to predict low-dose RBEs based on fits to the initial or low-dose slopes of dose-response data. Data fitted included chromosome aberrations, oncogenic transformations, and cytotoxicity in mammalian cells. From those efforts, extrapolated low-dose RBE values ranging in magnitude from 10 to 50 (e.g., see NCRP Figures 2.10 and 3.8) were common. In contrast to the NCRPs low-dose estimates, RBEs that we have computed from the biologically-based cell-kinetics model for cytopenia and cell proliferation (both closely related to chromosome aberrations and cytotoxicity) are significantly less than 10, even at 0.01 Gy) and seem much closer to experimentally derived values that were actually observed from different lineages of mammalian cells tested by in vitro bioassays (e.g., see NCRP Figures 2.11 and 3.13). In addition, our computed RBEs seem quite close to measured values for cultured cells of human kidney origin--see Figure 1 of (Barendsen 1971). Most published RBE values below about 0.20 Gy are estimated based on extrapolations below the range of accurate experimental measurements. Although the NCRP linear-quadratic model and our cell kinetics model show remarkable contrast at low dose, both fit experimental data accurately at high doses and produce similar estimates for the high-dose RBEs.

Table 4-1. Summary of mouse mortality experiments with fast neutrons from which the cell-kinetics model parameters for 1-hit cell killing were evaluated.

Item	Stewart et al. (1982)	Delihias and Curtis (1958)	Vogel et al. (1957)	Upton et al. (1956)	Carter et al. (1956)	Strike (1969)	Strike (1969)
Mouse strain	C57BL/6xCBA	CF1	CF1	RF	CF1	C3H	C57BL
Neutron source	Triga	<sup>235</sup> U target	Argonne CP-3'	Be target	Be target	D-T	D-T
Neutron:gamma (dose or kerma)	30:1 (8" of Pb)	93:7	93:7	6:1	9:1	negligible $\gamma$	negligible $\gamma$
Neutron energy (or quality)	0.7 MeV	49 keV/ $\mu$ m	fission (9 cm of Pb)	48 keV/ $\mu$ m	9 MeV	14 MeV	14 MeV
Neutron rate (Gy min <sup>-1</sup> )	0.40	0.21	0.001-0.03	0.67-1.25	0.80	0.03-0.50	0.03-0.50
Neutron dose (Gy)	3.25-4.75	3.14-4.52	1.54-2.69	1.93-5.65	2.67-4.83	2.82-7.07	3.43-6.01
# Groups (neutrons)	7	7	20	11	10	15	6
Group size (neutrons)	20-180	20-26	22-229	5-25	22-24	10-70	39-80
Photon source	<sup>60</sup> Co	250 kVp	<sup>60</sup> Co	250 kVp (& <sup>60</sup> Co)	250 kVp	250 kVp	250 kVp
Photon rate (Gy min <sup>-1</sup> )	0.40	1.9	0.0076-0.11	0.76 (0.60)	0.25	0.21	0.21
Photon dose (Gy)	7.0-10.0	4.75-6.24	11.7-14.3	4.4-9.16	3.53-6.42	3.78-8.64	4.32-8.10
# Groups (photons)	7	10	21	5 (8)	12	9	6
Group size (photons)	36-40	10-80	24-164	10-24	19-20	22-50	30-50

Table 4-2. Rate constants for 1-hit killing of cells that are critical to compensatory myelopoiesis by fast neutrons are given in column 7. Each  $\lambda_{NK}$  value uniquely determines a neutron cell-kinetics model because only a  $\lambda_{NK}$  and a species-specific proliferation constant are required for the two-coefficient stromal-cell model.

Investigator:	Neutron-Source: <sup>2</sup>	Rate (Gy min <sup>-1</sup> )	N-LD <sub>50</sub> (Gy):	X/Y-Source:	X/Y-LD <sub>50</sub> (Gy):	$\lambda_{NK}$ (Gy <sup>-1</sup> ):	Strain/Comments:
<b>Fission: Animal mortality</b>							
Vogel et al. (1957)	<sup>235</sup> U + 9 cm Pb	0.001-0.03	2.10	<sup>60</sup> Co	8.8	1.12	CF <sub>1</sub>
Delhas and Curtis (1958)	<sup>235</sup> U 1 MeV (49 keV/ $\mu$ m)	0.21	3.66	250 kVp	6.36	0.629	CF <sub>1</sub> ; 7% $\gamma$
Stewart et al. (1982)	triga + 8" of Pb (0.7 MeV)	0.40	3.71	<sup>60</sup> Co	8.79	0.623	hybrid; 3.3% $\gamma$
Upton et al. (1956)	22 MeV P on Be 1 MeV (48 keV/ $\mu$ m)	0.67-1.25	3.62	<sup>60</sup> Co/250 kVp	6.9/5.0	0.544	RF; 16% $\gamma$
<b>Accelerator/cyclotron:</b>							
Carter et al. (1956)	20 MeV D on Be 9 MeV	0.80	3.15/3.50 Males/Females	250 kVp	4.9/5.7 Males/Females	0.580	CF <sub>1</sub> ; 10% $\gamma$
Strike (1969)	14 MeV	0.03-0.50	4.32	250 kVp	6.80	0.556	C57BL
Strike (1969)	14 MeV	0.03-0.50	4.80	250 kVp	7.04	0.489	C3H
<b>Fission: CFU-F/Stroma<sup>3</sup></b>							
Meijne et al. (1992)	<sup>235</sup> U; mean = 1 MeV	0.10	CFU-F	0.75	1	1.3	Femur to Cult.; 9% $\gamma$
Meijne et al. (1992)	<sup>235</sup> U; mean = 1 MeV	0.10	Ect. femur	0.84	1	1.2	support CFU-C; 9% $\gamma$
Meijne et al. (1992)	<sup>235</sup> U; mean = 1 MeV	0.10	Ect. femur	0.92	1	1.1	support CFU-S; 9% $\gamma$
Meijne et al. (1992)	<sup>235</sup> U; mean = 1 MeV	0.10	Ect. spln	1.49	1	0.67	support CFU-C; 9% $\gamma$
Chertkov and Gurevitch (1979)	Obninsk R. (0.85 MeV)	1.28	Mouse stroma	1.61	1.4	0.55	Renal Capsule; 5% $\gamma$
Meijne et al. (1992)	<sup>235</sup> U; mean = 1 MeV	0.10	Ect. spln	2.29	1	0.44	Support CFU-S; 9% $\gamma$
14 MeV: CFU-F Xu et al. (1983)	14.7 MeV	0.20-0.30	CFU-F	1.36	- 1.0	0.73	Femur to Culture

<sup>2</sup> P = Proton; D = Deuterium; Ect. = ectopic transplant; spln = spleen; Dif = diffusion; and Cult = culture. <sup>3</sup>D<sub>0</sub> and  $\lambda$  may reflect renewing vs. functional stroma.



Table 4-3. Dose-Rate RBEs were computed for marrow stromal cells. Values outside parentheses were based on how different irradiation patterns affect the nadir (i.e., cytopenia) of the human stroma-cell survival curve. Values inside the parentheses are for compensatory cellular proliferation (above normal homeostatic demand) and were computed from ratios of cells killed at a particular dose (for 6 dose rates) of the test radiation divided by the cells killed by an equal prompt dose of  $^{60}\text{Co}$ .

**I. RBEs for Fission energy neutrons at different doses and dose rates compared with pulse irradiation by  $^{60}\text{Co}$ .**

Dose (Gy) <sup>a</sup>	EXPOSURE PERIOD				
	1 minute	1 hour	1 day	1 week	1 month
0.01	4.24 (4.24)	4.23 (4.24)	4.00 (4.24)	2.89 (4.24)	1.16 (4.25)
0.05	4.06 (4.06)	4.05 (4.06)	3.84 (4.07)	2.80 (4.08)	1.15 (4.10)
0.10	3.89 (3.89)	3.88 (3.89)	3.68 (3.90)	2.70 (3.92)	1.13 (3.96)
0.25	3.54 (3.54)	3.53 (3.54)	3.36 (3.55)	2.49 (3.60)	1.07 (3.70)
0.50	3.20 (3.20)	3.19 (3.20)	3.04 (3.22)	2.26 (3.31)	1.00 (3.50)
1.00	2.85 (2.85)	3.20 (3.20)	2.70 (2.88)	2.01 (3.08)	0.91 (3.50)
1.50	2.65 (2.65)	2.65 (2.65)	2.52 (2.71)	1.88 (3.04)	0.85 (3.97)
2.00	2.53 (2.53)	2.53 (2.53)	2.40 (2.61)	1.80 (3.13)	0.81 (6.88)
2.50	2.44 (2.44)	2.44 (2.44)	2.32 (2.55)	1.73 (3.44)	0.78 ( <sup>b</sup> )
3.00	2.37 (2.37)	2.37 (2.37)	2.26 (2.52)	1.69 (4.51)	0.75 ( <sup>b</sup> )
3.50	2.32 (2.32)	2.32 (2.32)	2.21 (2.51)	1.67 ( <sup>b</sup> )	0.73 ( <sup>b</sup> )
4.00	2.28 (2.28)	2.28 (2.28)	2.18 (2.52)	1.50 ( <sup>b</sup> )	0.72 ( <sup>b</sup> )
4.50	2.24 (2.24)	2.24 (2.24)	2.15 (2.56)	1.64 ( <sup>b</sup> )	0.70 ( <sup>b</sup> )

**II. RBEs for Fusion energy neutrons at different doses and dose rates compared with pulse irradiation by  $^{60}\text{Co}$ .**

0.01	3.13 (3.13)	2.12 (3.13)	2.95 (3.13)	2.13 (3.13)	0.86 (3.14)
0.05	3.03 (3.03)	3.02 (3.03)	2.86 (3.03)	2.08 (3.04)	0.85 (3.05)
0.10	2.93 (2.93)	2.92 (2.93)	2.77 (2.93)	2.02 (2.94)	0.84 (2.97)
0.25	2.70 (2.70)	2.70 (2.70)	2.56 (2.71)	1.89 (2.74)	0.81 (2.79)
0.50	2.47 (2.47)	2.47 (2.47)	2.34 (2.48)	1.74 (2.53)	0.76 (2.63)
1.00	2.21 (2.21)	2.21 (2.21)	2.10 (2.23)	1.56 (2.33)	0.70 (2.54)
1.50	2.06 (2.06)	2.06 (2.06)	1.96 (2.09)	1.46 (2.25)	0.66 (2.62)
2.00	1.96 (1.96)	1.96 (1.96)	1.86 (2.00)	1.39 (2.23)	0.63 (2.89)
2.50	1.89 (1.89)	1.89 (1.89)	1.79 (1.94)	1.33 (2.27)	0.60 (3.69)
3.00	1.83 (1.83)	1.83 (1.83)	1.74 (1.90)	1.30 (2.38)	0.58 ( <sup>b</sup> )
3.50	1.79 (1.79)	1.79 (1.79)	1.70 (1.87)	1.27 (2.59)	0.57 ( <sup>b</sup> )
4.00	1.75 (1.75)	1.75 (1.75)	1.67 (1.85)	1.25 (3.14)	0.55 ( <sup>b</sup> )
4.50	1.72 (1.72)	1.72 (1.72)	1.64 (1.85)	1.23 ( <sup>b</sup> )	0.54 ( <sup>b</sup> )

<sup>a</sup> Dose to marrow.

<sup>b</sup> Chronic exposure can kill more cells than present at any time—equality between the EPD and chronic exposure is not possible.

Table 4-4. Rate constants for 1-hit killing of hematopoietic CFU-S and CFU-C cells by fast neutrons are given in column 7. Each  $\lambda_{NK}$  value uniquely defines a new neutron cell-kinetics model because only a  $\lambda_{NK}$  and a species-specific proliferation constant are required for the two-coefficient stem-cell model.

Investigator:	Neutron Source:	Rate (Gy <sup>-1</sup> min <sup>-1</sup> )	Assay:	D <sub>0</sub> (Gy <sup>-1</sup> ):	n:	$\lambda_{NK}$ (Gy <sup>-1</sup> ):	Comment:
<b>Fission: CFU-S</b>							
Ainsworth et al. (1969)	triga	0.40	CFU-S	0.42	1.5	2.2	Spleen to Spln; 20% $\gamma$
Davids (1973)	<sup>235</sup> U; mean = 1 MeV		Transpl.	0.45	1	2.2	Rescue of CBA; 9% $\gamma$
Boyum et al. (1978)	<sup>235</sup> U; mean = 1 MeV	na	CFU-C	na	1	2.1	Dif-Chamb; 15% $\gamma$
Carsten et al. (1976)	<sup>235</sup> U	0.20	CFU-S	0.5078	1	2.0	Femur to Spln; 15% $\gamma$
Carsten et al. (1976)	<sup>252</sup> Cf	0.0018	CFU-S	0.5033	1	2.0	Femur to Spleen
<b>Accelerator/cyclotron: CFU-S</b>							
Carsten et al. (1976)	0.43 MeV	0.0081	CFU-S	0.2814	1	3.6	Femur to Spleen
Boyum et al. (1978)	0.44 MeV	0.03	CFU-C	na	1	3.4	Dif-Chamb; 1.9% $\gamma$
Carsten et al. (1976)	0.66 MeV	0.0093	CFU-S	0.3450	1	2.9	Femur to Spleen
Carsten et al. (1976)	1.0 MeV	0.0078	CFU-S	0.2812	1	3.6	Femur to Spleen
Carsten et al. (1976)	1.5 MeV	0.0093	CFU-S	0.2880	1	3.5	Femur to Spleen
Carsten et al. (1976)	1.8 MeV	0.0078	CFU-S	0.2962	1	3.4	Femur to Spleen
Ainsworth et al. (1969)	mean = 2.5 MeV	0.06	CFU-S	0.51	1.2	1.9	Spleen to Spleen
Carsten et al. (1976)	5.7 MeV	0.012	CFU-S	0.6962	1	1.4	Femur to Spleen
Boyum et al. (1978)	6.05 MeV	0.09	CFU-C	na	1	2.1	Dif-Chamb; 6% $\gamma$
Carsten et al. (1976)	13.4 MeV	0.018	CFU-S	0.9459	1	1.1	Femur to Spleen
Hendry and Howard (1971)	14 MeV	0.15	CFU-S	0.581	1.7	1.5	Femur to Spleen
Broerse et al. (1971)	14 MeV (Manchester)	0.08	CFU-S	0.625	1.34	1.5	Femur to Spleen
Duncan et al. (1969)	14 MeV	0.08	CFU-S	0.637	1.38	1.5	Femur to Spleen
Ainsworth et al. (1969)	14.7 MeV	0.30	CFU-S	0.63	1.6	1.4	Spleen to Spleen
Broerse et al. (1978)	14 MeV (Rijswijk)	0.15	CFU-S	0.783	1.34	1.2	Femur to Spleen
Boyum et al. (1978)	15 MeV	0.05	CFU-C	na	1	0.94	Dif-Chamb; 5% $\gamma$

Table 4-5. Dose-Rate RBEs were computed for hematopoietic stem cells. Values outside parentheses were computed based on how different irradiation patterns affect the nadir of the human stem-cell survival curve. Values inside the parentheses are for compensatory cellular proliferation (above normal homeostatic demand) and were computed from ratios of cells killed at a particular dose (for 6 dose rates) of the test radiation divided by the cells killed by an equal prompt dose of  $^{60}\text{Co}$ .

**I. RBEs for Fission energy neutrons at different doses and dose rates compared with pulse irradiation by  $^{60}\text{Co}$ .**

Dose (Gy) <sup>a</sup>	EXPOSURE PERIOD				
	1 minute	1 hour	1 day	1 week	1 month
0.01	5.02 (5.02)	4.99 (5.02)	4.44 (5.02)	2.39 (5.04)	0.72 (5.05)
0.05	3.35 (3.35)	3.34 (3.35)	2.98 (3.37)	1.69 (3.41)	0.60 (3.46)
0.10	2.82 (2.82)	2.81 (2.82)	2.49 (2.84)	1.40 (2.93)	0.52 (3.02)
0.25	2.35 (2.35)	2.34 (2.35)	2.04 (2.41)	1.09 (2.66)	0.41 (2.94)
0.50	2.16 (2.16)	2.14 (2.16)	1.86 (2.31)	0.93 (3.13)	0.34 (4.99)
1.00	2.06 (2.06)	2.06 (2.06)	1.78 (2.55)	0.84 ( <sup>b</sup> )	0.28 ( <sup>b</sup> )
1.50	2.03 (2.03)	2.03 (2.03)	1.79 ( <sup>b</sup> )	0.84 ( <sup>b</sup> )	0.25 ( <sup>b</sup> )
2.00	2.01 (2.01)	2.01 (2.01)	1.80 ( <sup>b</sup> )	0.88 ( <sup>b</sup> )	0.24 ( <sup>b</sup> )
2.50	2.00 (2.00)	2.00 (2.00)	1.82 ( <sup>b</sup> )	0.93 ( <sup>b</sup> )	0.23 ( <sup>b</sup> )
3.00	1.99 (1.99)	1.99 (1.99)	1.83 ( <sup>b</sup> )	1.00 ( <sup>b</sup> )	0.22 ( <sup>b</sup> )
3.50	1.99 (1.99)	1.99 (1.99)	1.84 ( <sup>b</sup> )	1.07 ( <sup>b</sup> )	0.22 ( <sup>b</sup> )
4.00	1.99 (1.99)	1.99 (1.99)	1.86 ( <sup>b</sup> )	1.13 ( <sup>b</sup> )	0.22 ( <sup>b</sup> )
4.50	1.98 (1.98)	1.98 (1.98)	1.86 ( <sup>b</sup> )	1.19 ( <sup>b</sup> )	0.22 ( <sup>b</sup> )

**II. RBEs for Fusion energy neutrons at different doses and dose rates compared with pulse irradiation by  $^{60}\text{Co}$ .**

0.01	3.78 (3.78)	3.76 (3.78)	3.34 (3.78)	1.76 (3.79)	0.52 (3.80)
0.05	2.58 (2.58)	2.57 (2.58)	2.30 (2.58)	1.30 (2.61)	0.44 (2.63)
0.10	2.15 (2.15)	2.14 (2.15)	1.91 (2.16)	1.08 (2.21)	0.39 (2.25)
0.25	1.75 (1.75)	1.74 (1.75)	1.53 (1.78)	0.84 (1.89)	0.32 (2.01)
0.50	1.57 (1.57)	1.56 (1.57)	1.36 (1.63)	0.70 (1.92)	0.26 (2.29)
1.00	1.47 (1.47)	1.47 (1.47)	1.27 (1.64)	0.61 (4.32)	0.21 ( <sup>b</sup> )
1.50	1.44 (1.44)	1.44 (1.44)	1.25 (1.83)	0.59 ( <sup>b</sup> )	0.19 ( <sup>b</sup> )
2.00	1.42 (1.42)	1.42 (1.42)	1.25 ( <sup>b</sup> )	0.59 ( <sup>b</sup> )	0.18 ( <sup>b</sup> )
2.50	1.41 (1.41)	1.41 (1.41)	1.25 ( <sup>b</sup> )	0.60 ( <sup>b</sup> )	0.17 ( <sup>b</sup> )
3.00	1.41 (1.41)	1.41 (1.41)	1.26 ( <sup>b</sup> )	0.62 ( <sup>b</sup> )	0.16 ( <sup>b</sup> )
3.50	1.40 (1.40)	1.40 (1.40)	1.27 ( <sup>b</sup> )	0.65 ( <sup>b</sup> )	0.16 ( <sup>b</sup> )
4.00	1.40 (1.40)	1.40 (1.40)	1.28 ( <sup>b</sup> )	0.68 ( <sup>b</sup> )	0.16 ( <sup>b</sup> )
4.50	1.39 (1.39)	1.39 (1.39)	1.29 ( <sup>b</sup> )	0.71 ( <sup>b</sup> )	0.15 ( <sup>b</sup> )

<sup>a</sup> Dose to marrow.

<sup>b</sup> Chronic exposure can kill more cells than present at any time—equality between the EPD and chronic exposure is not possible.

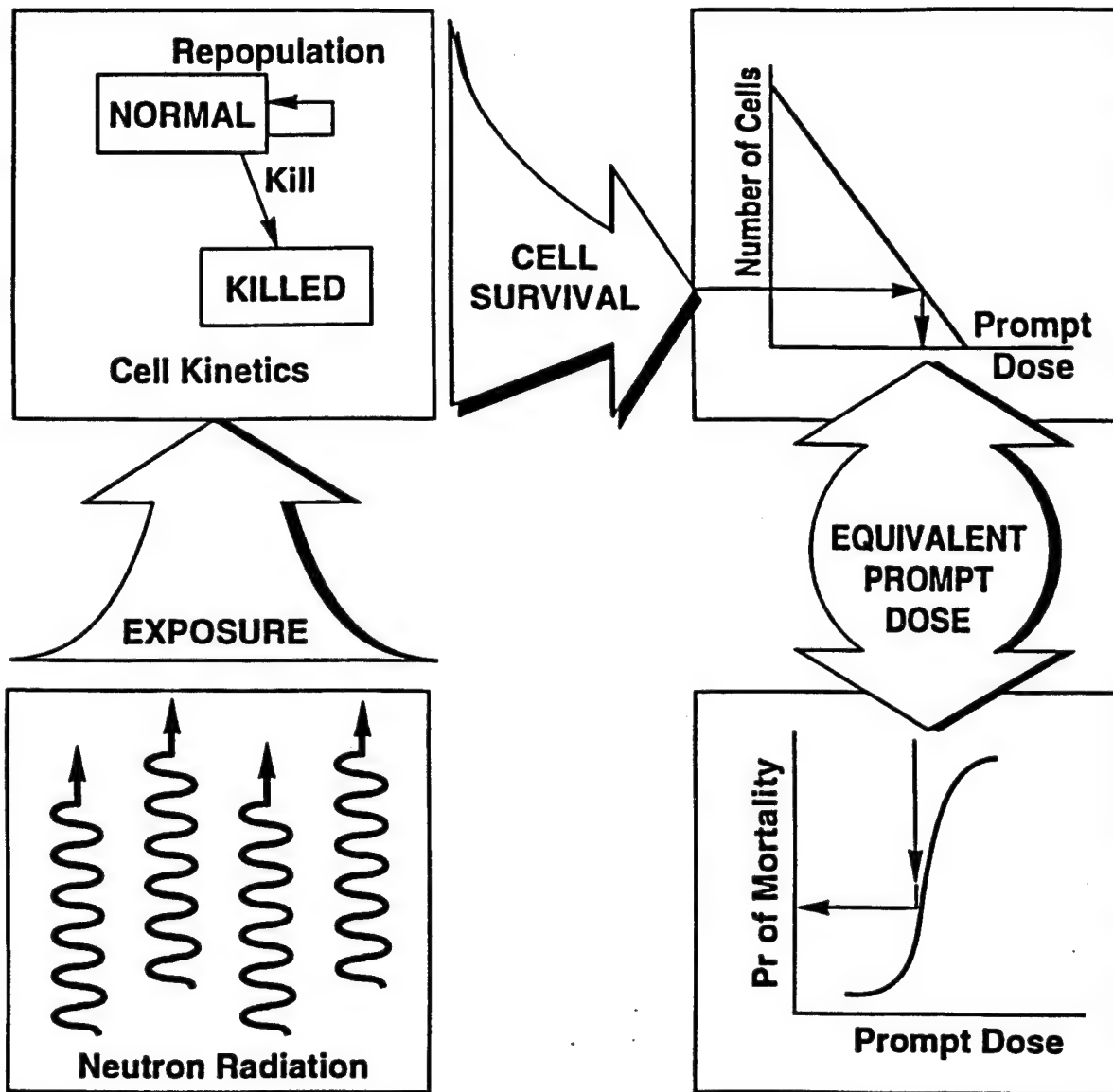


Figure 4-1. Schematic of the joint cell-kinetics and animal mortality models used for neutron exposures. Compare with the photon model shown in Figure 1-2.

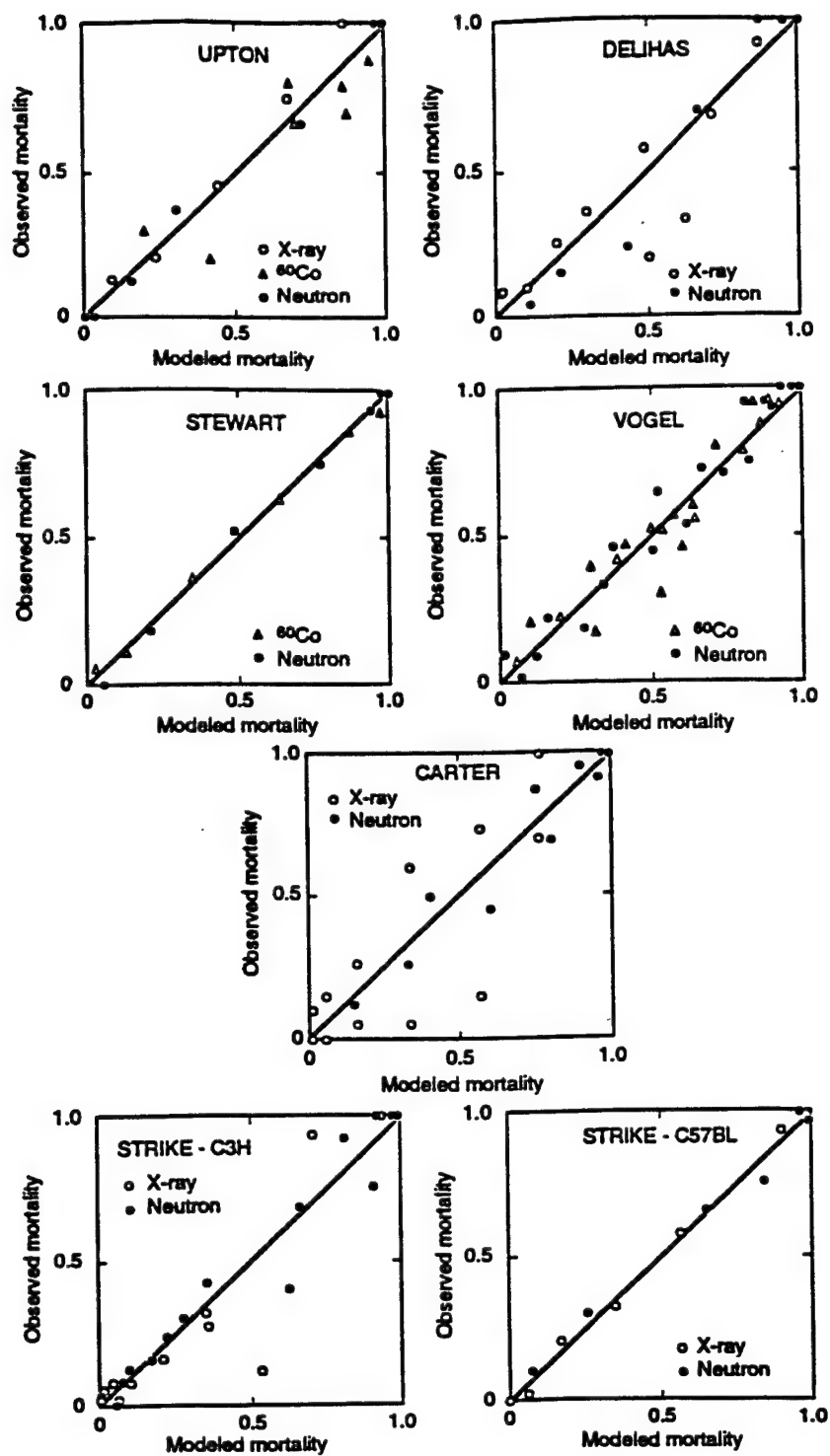


Figure 4-2. Scatterplots of observed experimental mortality vs model predictions.

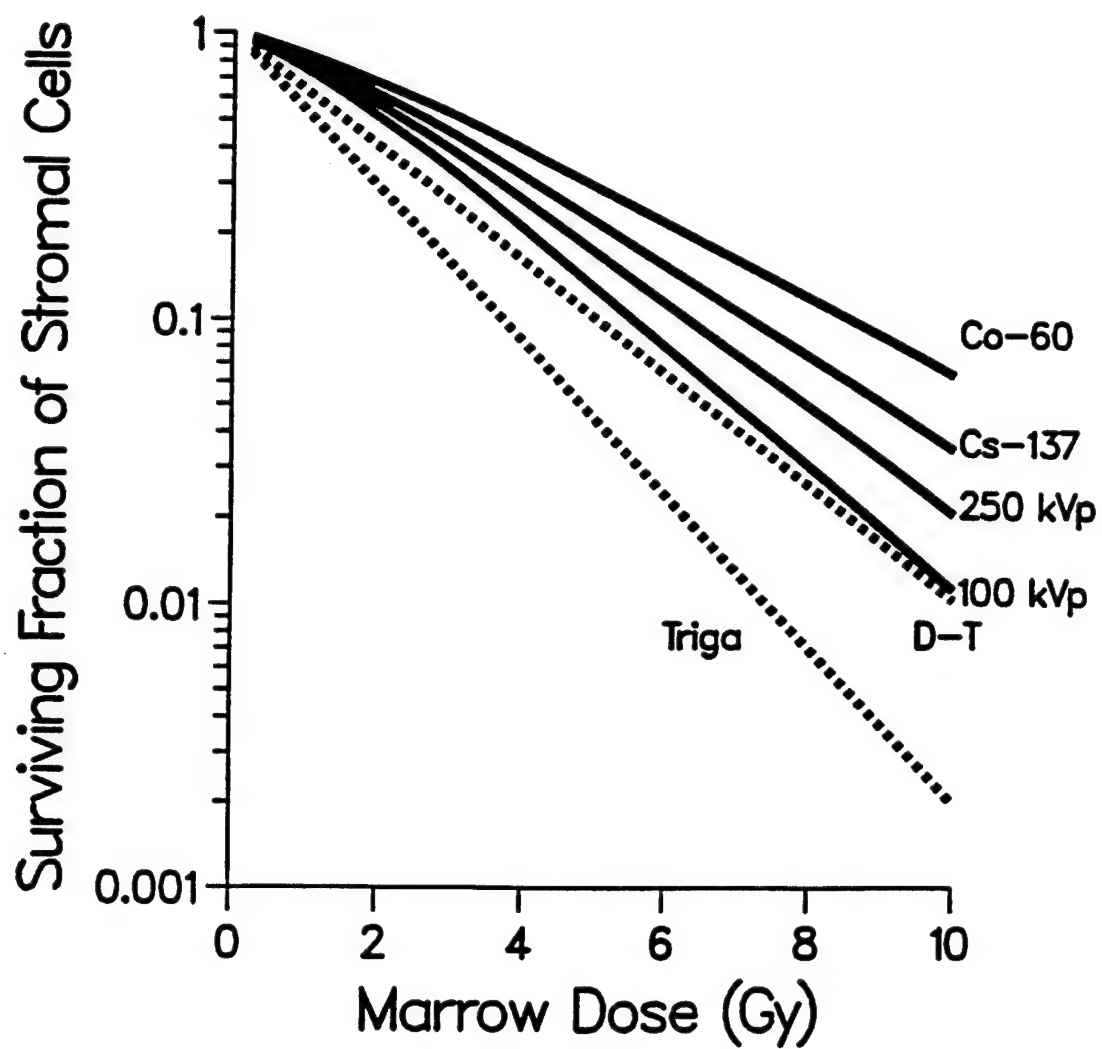


Figure 4-3. Stromal cell survival vs prompt dose of different radiations.

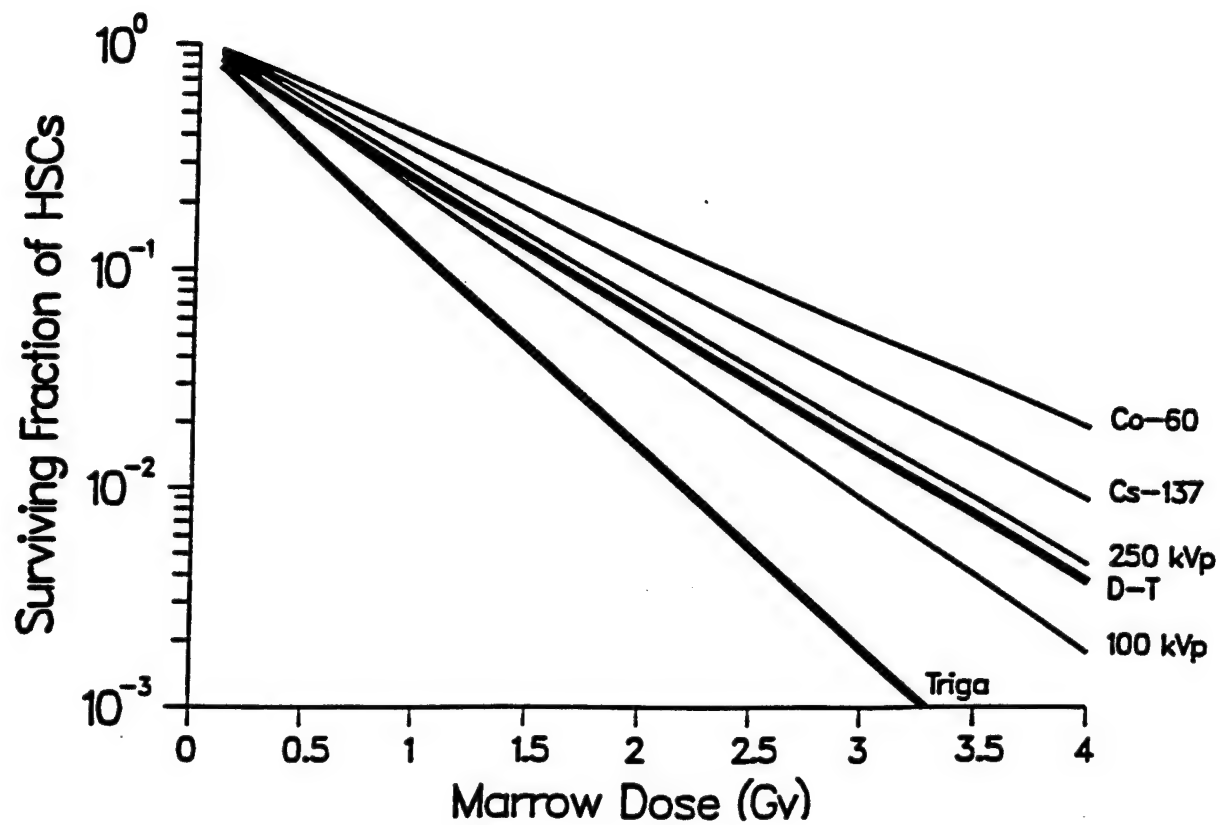


Figure 4-4. Stem cell survival vs prompt dose of different radiations.

## SECTION 5

### HUMAN LEUKEMIA AND LYMPHOMA

#### 5.1 CELL KINETICS RATE CONSTANTS FOR LEUKEMIA/ LYMPHOMA

The active marrow has been described as a union of lymphoreticular and differentiated myeloid tissue (Aleksandrowicz et al. 1965). Both tissues derive from a common, primitive stem cell, although lymphatic tissues may complete differentiation pathways outside of the marrow, e.g. production of T-lymphocytes in the thymus gland. The pattern of myeloid leukemia seems to be consistent with the thesis (Sherr 1987) that the commitment of pluripotent myeloid cells to different hematopoietic lineages appears to be stochastic and depends upon the expression of receptors for hematopoietic colony-stimulating factors which regulate cell proliferation, differentiation, and survival.

Myeloid leukemia consists of proliferation of myelopoietic cells in the marrow and extramedullary sites. The marrow cavity becomes packed with abnormal myelocytes which replace nearly all of the normal myelopoietic tissues. The predominant cell of leukemia is usually of the neutrophilic series, although early in the disease pathology there may be an excess of all granulocytic forms, platelets, and erythrocytes. Later, the replacement of marrow by leukemic cells results in anemia and bleeding. Cause of death for acute leukemia and myelodysplastic syndrome is usually from infection and/or hemorrhage (Clarkson and Rubinow 1977; Coutinho et al. 1990) exactly as in the acute radiation syndrome for which the cell-kinetics model based on the "critical cell" concept was originally developed. Time of death usually occurs soon after the leukemia blood-cell count has passed  $10^{12}$  (Clarkson and Rubinow 1977).

Bone marrow stromal cells may be involved in hematopoietic malignancies. As described by (Dilly and Jagger 1990), *"Interest in the way in which stromal cells control haemopoiesis naturally leads on to their possible role in haematological malignancies. Several oncogene protein products are related to growth factors so, theoretically, oncogenes acting through marrow stromal cells might induce abnormal proliferation of haemopoietic cells, predisposing them to malignant change. . . (stromal cells) might be important in maintaining a suitable microenvironment for the growth of malignant haemopoietic cells similar to their interaction with benign haemopoietic cells."*



The granulocyte-macrophage colony-stimulating factors (i.e., GM-CSF's) are of widespread interest as radioprotectants and as a means to mediate the undesirable effects of accidental and therapeutic irradiations. A few years ago, Robert Gale administered GM-CSF agents to radiation accident victims. His usage was somewhat controversial within the medical community because it preceded the formal FDA approval that is usually required for human applications. It does appear, however, that GM-CSF inducers may be potentially very useful to alleviate the untoward effects from human exposures. Clearly, GM-CSF's have demonstrated a capacity to reduce acute mortality in test animals.

The GM-CSF studies may also help to reveal the etiological mechanisms of leukemia and other hematopoietic neoplasms. For example, two types of control over the GM populations have become evident. In one, the basal populations of cells could be maintained by CSF production by stromal cells within the myelopoietic marrow--which corresponds to a system that appears to be able to repopulate cells in a compensatory manner following toxic irradiations (Metcalf 1985). The second type of homeostatic control involves tissues throughout the body and can be activated by microorganisms and/or their products such as endotoxin. In the case of ionizing radiations, depopulation of myelopoietic cells results from two fundamental processes: inactivation of the radiosensitivity progenitor cells, and killing of differentiated (but highly radiosensitive cells) such as lymphocytes. Injury to barrier membranes of the body, sublethal injury of cells, and autolysis of killed cells may all result in the body being exposed to greater levels of toxic microbes and cytotoxins. Thus, for ionizing radiations, the two processes are concomitant and do not occur singularly.

According to the excellent review by (Metcalf 1985),

*"Myeloid leukemias are clonal neoplasms of granulocytes-macrophage precursor cells. A current popular view of the cancerous state is that the uncontrolled proliferation exhibited by a cancer cell population is ascribed to the action of viral or cellular oncogenes whose products are either specific growth factors or receptors for such factors. Cancer cell proliferation is viewed, not as an autonomous process, but as the response of cells to oncogene-induced autosynthesis of specific growth factors or receptors. It is postulated that the polypeptides involved could in some cases be structurally normal with a cellular oncogene becoming dysregulated by translocation or activation by viral enhancers, while in other situations the oncogene may be structurally abnormal because of mutational events.*

*Since the CSF's are the only known proliferative stimuli for granulocyte and macrophages, it is of interest to determine whether the myeloid leukemias are ascribable to oncogene-deranged autosynthesis of CSF or CSF receptors. There is no sequence homology between the CSF's and the known oncogenes, but no sequence data are yet available for the CFS receptors.*

*Is there any evidence that myeloid leukemia cells are autostimulating because of an acquired capacity to synthesize their own CSF's? Data on this question are extensive and quite unambiguous for leukemia cells from patients with either acute or chronic myeloid leukemia. In no instance has it been documented that myeloid leukemia cells are capable of sustained autonomous proliferation in vitro. The proliferation . . . is like normal cells, absolutely dependent on the addition of exogenous CSF, and the concentrations of CSF required are similar to those required to stimulate the proliferation of normal cells. . . The absolute levels of CSF that can be produced by an emerging leukemia clone of a few cells would be insignificant compared with the CSF normally produced by adjacent stromal cells or with the amount reaching the cells via the circulation, and it is improbable that autoproduct of CSF by leukemia cells would represent a significant event in the emergence of a leukemia clone (Note: Two years after Metcalf's review, Sherr (1987) noted "most human leukemia cells depend on CSFs for growth in culture. Even under the best circumstances, cell lines can be established from only a small percentage of patients with acute leukemia in spite of their cells' proliferative potential in vivo.")*

*Taken together, the data on the lack of autonomous growth ability and the unremarkable receptor numbers on leukemic cells argue strongly against a simple autocrine growth model for myeloid leukemia cells. A more likely possibility, regardless of whether or not leukemic cells can synthesize CSF, is that an intrinsic abnormality exists in leukemic cells as a consequence of which CSF-stimulated proliferation results in an abnormally high ratio of self-generative divisions versus divisions leading to the production of differentiating progeny. In this model, CSF would be a necessary cofactor in the emergence of a leukemic clone since it is mandatory for cell proliferation, but an oncogene product (sic., or reaction processes and/or products resulting from ionizing radiations), would be responsible for the aberrant pattern of resulting cell divisions."*

Recognizing that almost any population subgroup—whether it be human or animal—has a spectrum of exposures to cellular "initiators" of leukemia through heredity, natural background radiations, medical diagnostic exposures, environmental chemical compounds such as benzene, etc. there seems to be no

unique, deterministic role for cellular initiation or neoplastic transformation in the disease etiology. In contrast, our analytical approach (Jones et al. 1983; Jones 1984) has focused on the capacity of ionizing radiations and/or chemicals to affect the potentiation or promotion of the disease.

Most forms of human leukemias seem to begin with transformations of single, or at the most, a few hematopoietic cells and retain many of the properties of normal cells, including some aspects of proliferation. However, in frank leukemia, the leukemia cell population expands beyond homeostatic normality. For example, the blast cells of acute myeloblastic leukemia (AML) are maintained by myeloid stem cells (McCulloch et al. 1988). This continued expansion causes either replacement of the normal cells or inhibition of normal hematopoiesis because of excessive cellular density (Clarkson and Rubinow 1977). Cells may over concentrate in the marrow because they do not differentiate and migrate into peripheral blood. Over-population of leukemia cells in hematopoietic marrow seems to further reduce normal hematopoiesis. The type of leukemia that results may be determined primarily by which progenitor lineage hosts the initial transforming event and to a lesser degree by cytokines which direct differentiation of the "hit" cell. It is not always clear whether the parent leukemia stem cell is within the differentiation compartment that typifies the bulk of the aberrant cells or if the parent cell may reside in a more primitive precursor pool that is further restricted by cytokines (Clarkson and Rubinow 1977).

Early experiments demonstrated that injection of an average of only two viable leukemia cells was sufficient to produce leukemia in rodents (Hewitt 1958, Hewitt and Wilson 1959, Berry and Anderson 1961).

Thus, we conclude from Metcalf's review and other experimental results as summarized above, that whether the potentiating factors of leukemia derive from immunosuppression, compensatory myelopoiesis, stimulation of cytokines such as the GM-CSF's, or some combination of these processes, the bone-cell kinetics models which we have developed for marrow stromal cells (Jones et al. 1991; Morris et al. 1991, Morris et al. 1993) and for hematopoietic stem cells (Jones et al. 1993a) is suited to biologically based models for analysis of both animal and human data. Following are data which can be used to determine the rate constants in the cell kinetics link of the etiological model for radiogenic leukemias and lymphomas.

## 5.2 REPAIR.

It has been a generally accepted rule of radiation biology that cell killing of hematopoietic progenitor cells is independent of X-ray dose-rate from 0.05 to 0.25 Gy/min (FitzGerald et al. 1986). However to compare killing in this range with that from conventional 2 Gy/min irradiations, FitzGerald et al. (1986), measured cell killing in JY (B-Cell), Daudi (B-Cell), K45 (T-Cell), K562 (erythro), HL60 (monomyeloid), KG1 (monomyeloid), U937 (histiocytic/monocytic) human cell lines, and in bg/bg cl 1 mouse basophilic and rat LW12 acute myelogenous leukemias, and concluded that: *"Dose rate independent killing was demonstrated at several plating densities with mouse and rat leukemia lines and all human leukemia lines tested except lines HL60 and U937. With HL60, increased plating density increased the  $D_0$  at each dose rate. This effect was not attributable to an increased plating efficiency. With line U937 there was a clear dose-rate effect with increase in  $D_0$  from 88 rad,  $n$  4.6 at 200 rad/min, to  $D_0 = 166$ ,  $n$  2.3 at 5 rad/min. The data demonstrate that some human hematopoietic tumor derived cell lines of myeloid/monocytic/macrophage lineage can exhibit atypical repair of irradiation damage in vitro. This repair may be enhanced by conditions relevant to clinical TBI including low dose-rate and cell to cell interactions by tumor cells in close proximity."*

Some in vitro experiments have demonstrated that repair of sublethal damage does not seem to be of much importance for some leukemia cells. However, for normal bone marrow cells, a repair half-time of about 20 minutes has been estimated from biological experiments (Vegesna et al. 1985). From evaluation of the mouse data (Morris et al. 1991), maximum likelihood estimates of repair of sublethal injury to cells critical to hematopoietic survival gave a half-time of 43 minutes for therapeutic injury (or 21 minutes for dose near the  $LD_{10}$ ). From the composite analysis for mice, rats, dogs, sheep, swine, and burros, the values changed to 31 and 15 minutes, respectively (Morris et al. 1993). For hematopoietic stem cells we have used corresponding repair half-times of 6 and 12 minutes based on advice by Sinclair, because of the faster mitotic cycle (Jones et al. 1991). Two possibilities are suggested for the repair rate constant for a particular lineage of leukemia cells: either insignificant repair or alternatively repair postulated on inheritance from a stem or preleukemia lineage in which case the repair rate will be adjusted to the length of the mitotic cycle as described by (Jones et al. 1991).

## 5.3 PROLIFERATION.

"There appear to be no basic differences in the division cycles of normal and neoplastic cells except the normal cell may spend less time in certain phases of the cell cycle" (Clarkson and Rubinow 1977).

(O'Donoghue et al. 1987) used 2 and 4 days for leukemia doubling times. To establish bounding considerations for therapeutic schedules, they used a doubling time of 20 days.

The mean intermitotic time of CML myeloblasts is about 60 h or double that of normal myeloblasts (Clarkson and Rubinow 1977) which would seem to approximate the rate seen with a single therapeutic fraction used from marrow ablation, i.e., about 1.5 Gy of gamma photon radiation. Human promyelocytic leukemia line HL-60 has been measured to have a cell cycle time of 28-29 h (Laskin et al. 1991) which is near the value of  $26.7 \pm 2.2$  h that we estimated for stem cells under rapid growth kinetics (Jones et al. 1993a).

Most malignancies are monoclonal in nature, i.e. they derive from a single parent cell. Typically, first diagnosis is at about a billion cells (even for leukemias).  $2^{10} \sim 10^3$  so  $10^9$  equals  $2^{30}$ . Typically, death occurs at  $2^{40}$  (or  $10^{12}$ ) malignant cells and for leukemias this is generally 2 to 10 years post exposure. Thus, the effective doubling time is about  $(2 \times 365) / 40$  to  $(10 \times 365) / 40$  days or about 18 to 90 days. However, cells are constantly being lost--especially malignant cells from autonecrosis and inadequate vascularization. This loss fraction would vary with time and progression. The greatest effect would be when the malignancy expands to life-threatening size. If we assume that there is an average net loss of cells at 50% per cell generation, then  $1.5^x = 2^{40}$  would suggest that the gross number of doublings  $x = 68$ . If those 68 generations occurred over 2 years, the average doubling period would be 11 days; whereas, if the effect occurred over 10 years, the doubling would correspond to 54 days.

Because, leukemia cells are usually histologically linked to hematopoietic stem cells, one assumption can be to take an equal doubling as describe for HSCs at about 24 hours. This should describe the most rapid proliferation, especially late in the progression of the disease. Hence, assumed doubling times could be taken at 2, 4, 14, and 60 days, with the two shorter times perhaps corresponding to late pathological stages and compensatory repopulation between therapeutic fractions whereas, the longer times might correspond to early neoplastic development and could be used for assessing neoplastic risk associated with therapeutic procedures.

The form,  $S = 1 - [1 - \exp(-D/D_0)]^n$  has been used widely to describe the dose-response function for the surviving fraction of cells as a function of dose following exposure to ionizing radiations. In fact, this model has been used so extensively that experimental data have rarely been published--instead publications

have simply noted the values of  $n$  and  $D_0$  corresponding to particular experimental designs. Because the experimentally derived cell survival data of interest are not available, these two fitted constants from experimental data for CFU-S, CFU-F, and leukemia/lymphoma lineages can be used to describe the empirical behavior of the dose-response and that simulation in turn can be used to help estimate some of the numerical constants in our cell kinetics model that has a different algebraic form in order to model cellular processes of: (1) sublethal injury; (2) repair of sublethal injury; (3) killing of sublethally injured cells; (4) direct killing of phenotypically normal cells; and (5) compensatory repopulation in response to injury.

From data tabulated by (Potten and Hendry 1983) for 17 experiments in which CFU-S cells were irradiated in vivo with  $^{60}\text{Co}$  and  $^{137}\text{Cs}$ , the mean value of  $n$  was 1.22 with  $\sigma = 0.16$  for dose rates above 0.045 Gy/min. For CFU-F cell survival as reviewed by (Jones et al. 1993b), it was clear that those experiments similarly displayed an extrapolation number with a value near unity.

From Table 5-1, there are widely varying radiosensitivities for human leukemia and lymphoma cells irradiated with  $^{137}\text{Cs}$  at dose rates as shown by the 47 experiments using dose rates above 0.10 Gy/min. Experiments 53, 66, 71, and 72 had  $n$  values generally inconsistent with the other 43 experiments and were excluded as were values for X rays and  $^{60}\text{Co}$ . Of the remaining 43 curves for  $^{137}\text{Cs}$ , the radiosensitivities of the leukemia and lymphoma cells ranged from more sensitive than hematopoietic stem cells to more resistant than generally measured for CFU-F cells. Because the extrapolation numbers were in the general range of unity, the 43 curves were grouped into classes I to III according to whether: (I)  $D_0 < 1$  Gy; (II)  $1 \text{ Gy} \leq D_0 \leq 1.5$  Gy; or (III)  $D_0 > 1.5$  Gy to determine if  $n$  varied according to these classes. As seen in the following paragraph,  $n$  seemed to be quite constant and independent of the magnitude of  $D_0$ .

#### **5.4 RADIOSENSITIVITIES OF LEUKEMIA AND LYMPHOMA CELLS.**

Fourteen studies having a  $D_0 < 1$  Gy had a mean  $n$  of  $1.14 \pm 0.33$ ; twelve studies having a  $D_0$  between 1 and 1.5 Gy had a mean  $n$  of  $1.27 \pm 0.48$ ; and seventeen studies having a  $D_0 > 1.5$  Gy had a mean  $n$  of  $1.26 \pm 0.31$ .

Thus, it appears that a composite  $n$  value of 1.2 can be taken for the purpose of estimating cellular rate constants for reference malignant cell lines. That process was developed as follows:

## 5.5 RATE CONSTANTS FOR DIRECT KILLING OF "NORMAL MALIGNANT" CELLS.

A "normal malignant" cell is defined as a malignant cell without sublethal radiation injury. The several sets of experimentally derived data for malignant cell survival reflect widely variable degrees of radiosensitivity--in spite of having a remarkably constant extrapolation number  $n$ . The  $D_0$  values range from about 30 to 578. If the  $D_0$  values for the first 47 experiments (i.e.,  $^{137}\text{Cs}$  with dose rates above 10 cGy/min) are sorted according to radiosensitivities defined by the zones shown in Figure 5-1, the frequency of distribution associated with column 9 of Table 5-1, is: (S-3, 0), (S-2, 2), (S-1,6), (S,6), (S+1,6), (S+2,6), (F-1,16), (F,4), and (F+1,1), respectively according to classification zones shown in Figure 5-1. Therefore, four reference malignant cell lines will be hypothesized as listed in Table 1-6 based on  $D_0$  values of 0.73, 0.94, 1.23, and 1.96 Gy and  $n$  values of 1.2.

Table 5-1. Summary of radiosensitivities for leukemia and lymphoma cell lineages according to the dose-response formula given by  $y = 1 - (1 - e^{-x/D_0})^n$ .

Study:	Cell Line:	Leukemia (Lymphoma) Class	Source	Rate	$D_0$	n	Symbol	Class <sup>b</sup>
<i>Human Cells (<sup>137</sup>Cs):</i>								
(Johansson et al. 1982)	P3HR-1 (B-Cell)	Burkitt (Lymphoma)	<sup>137</sup> Cs	13	130	1.2	1	S+2
(Johansson et al. 1982)	U-698 (B-Cell)	Lymphatic (Lymphoma)	<sup>137</sup> Cs	13	180	1.2	2	F-1
(Johansson et al. 1982)	U-715 (B-Cell)	Lymphatic (Lymphoma)	<sup>137</sup> Cs	13	160	1.0	3	F-1
(Johansson et al. 1982)	SU-DHL-4(B-Cell)	Histiocytic (Lymphoma)	<sup>137</sup> Cs	13	140	1.2	4	S+2
(Ozawa et al. 1983)	Marrow	Acute Myelomonocytic	<sup>137</sup> Cs	131	210	1.3	5	F
(Ozawa et al. 1983)	Marrow	Acute Promyelocytic	<sup>137</sup> Cs	131	180	1.2	6	F-1
	Blood				195	1.0	7	F-1
(Ozawa et al. 1983)	Blood	Acute Myelomonocytic (R) <sup>c</sup>	<sup>137</sup> Cs	131	190	1.7	8	F-1
	Blood				185	1.1	9	F-1
(Ozawa et al. 1983)	Marrow	Acute Myelomonocytic	<sup>137</sup> Cs	131	140	1.4	10	F-1
	Blood				130	1.3	11	S+2
	Blood				100	1.1	12	S+1
(Ozawa et al. 1983)	Blood	Acute Myeloblastic	<sup>137</sup> Cs	131	135	1.3	13	S+2
(Ozawa et al. 1983)	Blood	Acute Myeloblastic (R)	<sup>137</sup> Cs	131	120	1.3	14	S+2
(Ozawa et al. 1983)	Marrow	Acute Myeloblastic	<sup>137</sup> Cs	131	90	1.2	15	S+1
(Ozawa et al. 1983)	Blood	Acute Myelomonocytic	<sup>137</sup> Cs	131	70	1.2	16	S
(Ozawa et al. 1983)	Blood	Acute Myelomonocytic	<sup>137</sup> Cs	131	70	1.1	17	S
(Ozawa et al. 1983)	Blood	Acute Myelomonocytic (R)	<sup>137</sup> Cs	131	55	1.0	18	S-1
(Ozawa et al. 1983)	Blood	Acute Myeloblastic	<sup>137</sup> Cs	131	50	1.0	19	S-1
(Ozawa et al. 1983)	Blood	Acute Myeloblastic (R)	<sup>137</sup> Cs	131	30	1.0	20	S-2
(Rhee et al. 1985)	HL-60	Acute Promyelocytic	<sup>137</sup> Cs	109.7	76.4	~9	21	S+1
(Uckun et al. 1991)	REH,Pre-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	65	1.1	22	S-1
(Uckun et al. 1991)	NALM-6,Pre-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	58.6	1.5	23	S-1
(Uckun et al. 1991)	KM-3,Pre-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	182.9	1.1	24	F-1
(Uckun et al. 1991)	HPB-Null,Pre-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	169.3	1.3	25	F-1
(Uckun et al. 1991)	NAMAL-WH,Early-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	169.1	1.0	26	F-1
(Uckun et al. 1991)	RAJI,Early-B	Acute Lymphoblastic	<sup>137</sup> Cs	109	144.4	1.3	27	F-1
(Uckun et al. 1988)	JC(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	177	2.1	28	F-1
(Uckun et al. 1988)	HD(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	83	3.8	29	S+1
(Uckun et al. 1988)	OC(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	128	2.6	30	F-1
(Uckun et al. 1988)	MS(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	50	0.8	31	S-1
(Uckun et al. 1988)	NF(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	450	1.1	32	F
(Uckun et al. 1988)	CH(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	101	1.0	33	S+1



Table 5.1 Summary of radiosensitivities for leukemia and lymphoma lineages according to the dose response formula given by  $y = 1 - (1 - e^{-x/D_0})^n$ . (Continued)

(Uckun et al. 1988)	AT(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	104	0.5	34	S+1
(Uckun et al. 1988)	JV(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	234	1.5	35	F
(Uckun et al. 1988)	SS(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	578	1.2	36	F+1
(Uckun et al. 1988)	JS(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	77	0.8	37	S
(Uckun et al. 1988)	AB(T-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	34	2.0	38	S-2
(Uckun et al. 1988)	KH(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	77	1.3	39	S
(Uckun et al. 1988)	PM(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	63	0.7	40	S-1
(Uckun et al. 1988)	BM(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	182	0.7	41	F-1
(Uckun et al. 1988)	CY(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	62	14.9	42	S
(Uckun et al. 1988)	KK(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	150	1.0	43	F-1
(Uckun et al. 1988)	KV(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	77	1.2	44	S
(Uckun et al. 1988)	BJ(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	161	1.4	45	F-1
(Uckun et al. 1988)	RR(B-Cell)	Acute Lymphoblastic	<sup>137</sup> Cs	100	359	1.6	46	F
				36.2	98.7	~5	47	S+2
				7.2	112.7	~4	48	-
				2.9	119.9	~3	49	-

**Human Cells (Other Radiations):**

(Welchselbaum et al. 1981)	45	Acute Lymphocytic	220 kV	80	147	1.1	50
(Welchselbaum et al. 1981)	K562	Erythroleukemia	220 kV	80	165	1.2	51
(Welchselbaum et al. 1981)	176	Acute Monomyelogenous	220 kV	80	76	4.0	52
(Welchselbaum et al. 1981)	HL60	Promyelocytic	220 kV	80	117	1.3	53
(FitzGerald et al. 1986)	KG1	Monomyeloid	250 kVp	5	81	2.04	54
				200	86	2.12	55
(FitzGerald et al. 1986)	HL60 <sup>a</sup>	Monomyeloid	250 kVp	5	129	2.32	56
				20	126	2.18	57
				200	125	2.20	58
(FitzGerald et al. 1986)	Daudi <sup>b</sup>	B-Cell (lymphoma)	250 kVp	5	57	1.54	59
				20	57	0.89	60
				200	57	1.32	61
(FitzGerald et al. 1986)	JY <sup>b</sup>	B-Cell (lymphoma)	250 kVp	5	61	1.84	62
				20	65	2.23	63
				200	58	2.32	64
(FitzGerald et al. 1986)	K562	Erythroleukemia	250 kVp	5	140	1.45	65
				20	140	1.68	66
				200	141	1.10	67

Table 5.1 Summary of radiosensitivities for leukemia and lymphoma lineages according to the dose response formula given by  $y = 1 - (1 - e^{-x/D_0})^n$ . (Continued)

(FitzGerald et al. 1986)	U937 <sup>c</sup>	Monocytic (lymphoma)	250 kVp 5	166	2.30	68
			20	128	4.40	69
			200	88	4.60	70
(FitzGerald et al.1986)	K45 <sup>d</sup>	T-Cell	250 kVp 5	58	1.30	71
			20	66	0.88	72
			200	61	0.80	73
(Morkovin et al. 1960)	Osgood	————	<sup>60</sup> Co —	140	2.	74
<b>Rat Cells:</b>						
(FitzGerald et al. 1986)	LW12	Acute Myelogenous	250 kVp 5	59	2.27	A
			200	56	1.80	B
<b>Mouse Cells:</b>						
(FitzGerald et al. 1986)	bg/bg	Basophillic	250 kVp 5	75	0.62	a
			20	64	1.08	b
			200	71	0.71	c
(Yau et al. 1979)	L5178Y-AIV	(lymphoma)	250 kVp 60	60	~1.35	d
(Yau et al. 1979)	L5178-S/F	(lymphoma)	250 kVp 60	220	5.5	e
(Hewitt and Wilson 1959)	CBA	Lymphocytic	<sup>60</sup> Co —	158	2.	f
(Bush and Brace 1964)	AKR/J	(lymphoma)	<sup>60</sup> Co 200	114	0.71	g
(Berry and Anderson 1961)	P-388;DBA/2	Lymphocytic	3 MeV X -	160	1.6	h

<sup>a</sup> granulocyte colonies; <sup>b</sup> lymphoid colonies; <sup>c</sup> monocyte-macrophage colonies; <sup>d</sup> lymphoma colonies; <sup>e</sup> Relapse;

<sup>b</sup> The classification of radiation sensitivity was made according to the zones illustrated in Figure 5-1.

# Human Cells: $^{137}\text{Cs}$

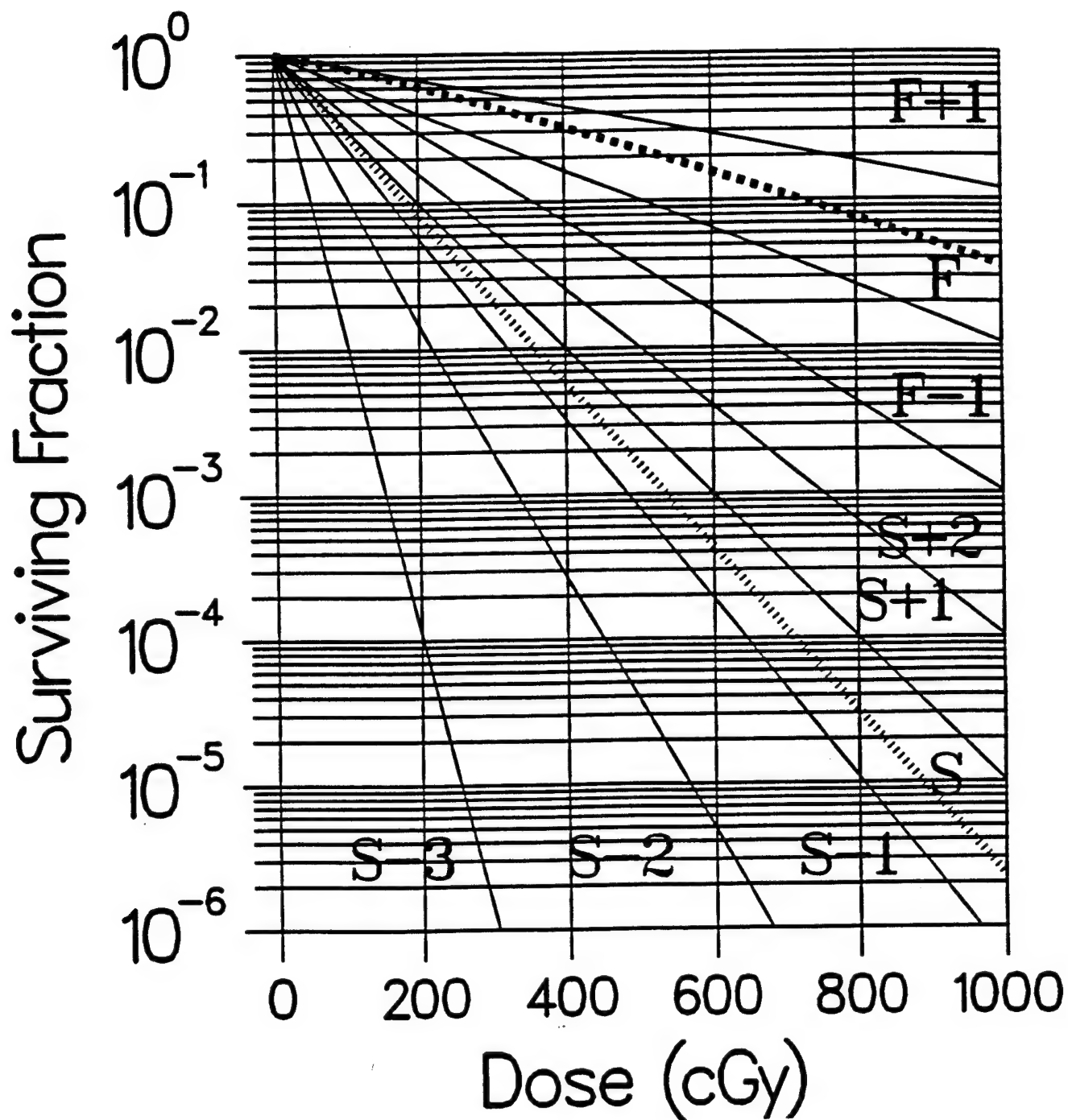


Figure 5-1. Zones used to classify the radiosensitivity of human leukemia and lymphoma cells exposed to  $^{137}\text{Cs}$ .

## SECTION 6

### MODEL VALIDATION

#### 6.1 INTRODUCTION AND BACKGROUND.

Consensus principles from radiation biology have been used to formulate a generic set of nonlinear, first-order, ordinary, differential equations for modeling of toxicity-induced compensatory cell kinetics in terms of sublethal injury, repair of sublethal injury, direct cytotoxicity, killing of cells with unrepaired sublethal injury, and repopulation in response to toxic injury. This cellular model was linked to a probit model of hematopoietic mortality that describes death from infection and/or hemorrhage between ~5 and 30 days. Mortality data from 27 experiments with 851 dose-response groups of test animals, in which doses were protracted by rate and/or fractionation, were used to simultaneously estimate all rate constants by maximum-likelihood methods (Morris et al. 1993). Data used represented 18,940 animals distributed according to: (mice, 12,827); (rats, 2,925); (sheep, 1,676); (swine, 829); (dogs, 479); and (burros, 204). Although a long-term, repopulating pluripotent hematopoietic stem cell is ancestral to all lineages needed to restore normal homeostasis, the dose-response data from the protracted irradiations indicate clearly that the particular rate-limiting lineage that is "critical" to hematopoietic recovery does not resemble stem-like cells with regard to radiosensitivity and repopulation rates.

Correspondences to Journals by (Hendry et al. 1994), (Roberts and Hendry 1994), and (Ruifrok and Thames 1994) criticized our conclusions that "*cells which regulate compensatory myelopoiesis correspond to marrow stromal or fibroblast cells with respect to radiosensitivity and doubling time*" (Cover Experimental Hematology, June 1993). Although, these conclusions are well supported by our publications, the criticisms were based mostly on: misinterpretation of our work, assumptions deriving from hypothetical tissue-rescuing units, and potential effects due to postulated cellular heterogeneity among marrow stem cells. We argued that whether or not these hypothetical constructs are biologically reasonable or not, those comments have no particular relevance to our published conclusions (Jones et al. 1994b, Morris et al. 1994a, Morris et al. 1994b), and in this brief communication we will use additional data from protracted irradiation protocols to gain further insight into the question "*Is hematopoiesis or its regulatory microenvironment the rate limiting factor for  $LD_{50/30}$ .*"

In addition to the model of compensatory hematopoiesis based on the generic "critical cell" approach and maximum likelihood estimation methods of animal mortality data, we used CFU-S experimental data to

evaluate a "stem-cell" kinetics model (in terms of those same non-specific damage, repair, and repopulation processes) for purposes of model comparisons, carcinogenic risk assessments, and optimization protocols for predictive models useful for considerations of marrow ablation and immunosuppression.

## **6.2 METHOD AND MATERIALS.**

### **6.2.1 Assumption: Acute Lethality Derives from a "Critical" Cell.**

When animals are irradiated, death from infection and/or hemorrhage may occur between about 5 and 30 days post-irradiation. The frequency of death can be described by a probit distribution function with fitted parameters comprised of the  $LD_{50}$  and slope (i.e., slope =  $\sigma^{-1}$  which is the inverse standard deviation of the frequency distribution). The  $LD_{50}$  and  $\sigma$  may be for the particular radiation field of interest or for a standard or reference radiation if there is a realistic way of modeling the underlying degree of cytopenia from the exposure of interest and converting that level of effect back to an equivalent reference dose of the standard radiation associated with the  $LD_{50}$  and  $\sigma$  estimates. Cytopenia of neutrophils and platelets is commonly accepted as the proximate cause of death, but the contributing cause of death could be inadequate cell populations related to [1] terminally differentiated cells themselves; [2] progenitor cells; or [3] progenitor-dependent lineages that interact with the undifferentiated pluripotent stem cells. For generality, the most sensitive cellular lineage in the complex multidimensional chain of compensatory hematopoiesis was analyzed generically and guided by MLE evaluations in contrast to more restrictive biological assumptions. One major advantage of this approach is that only one ( $LD_{50}$ ,  $\sigma$ ) combination was required for a complex experiment involving different: dose rates, exposure protocols, radiation sources, etc. (Morris et al. 1991 and 1993). In short, only changes with respect to the strain, species, cage care, and conditions of observation required additional  $LD_{50}$  and  $\sigma$  values. This treatment is in direct contrast to historical models that require new  $LD_{50}$ 's and  $\sigma$ 's when anything other than the magnitude of test dose was changed. One experiment in the analysis was comprised of 26 different  $LD_{50}$  protocols but all were consistent with a common  $LD_{50}$  and  $\sigma$  associated with an "equivalent prompt dose."

### **6.2.2 Generic Cell Model Evaluated from Mortality Data.**

Theory underlying the model and likelihood analysis have been described in the cited publications. Processes by which cells move among normal, injured, and killed populations are modeled by first-order, nonlinear equations.

### **6.2.3 Hematopoiesis Model Evaluated from CFU-S Data.**

The same functional form based on cellular damage, repair, and repopulation was evaluated for different numerical rate constants from experimental studies on CFU-S cells as described in a previous reference (Jones et al. 1993a). Damage and repair constants were estimated from dose-rate data of (Puro and Clark 1972) and their experiment is unique in this regard because five dose rates were used jointly for CFU-S survival studies and in parallel for animal mortality determinations. The proliferation constant was estimated from an analysis of published values obtained from an extensive literature review. The repair constant was taken from the evaluation described above for the lethality data base, but an additional normalization was applied according to the recommendation of Warren Sinclear that repair for a given type of cell could be taken as proportional to inter-mitotic cycle time (Jones et al. 1991).

### **6.2.4 Collection of Data Base for Comparisons.**

Thirty four experiments have been analyzed previously by maximum likelihood methods in order to estimate cellular rate constants within the model and are not considered suitable for model validation considerations--27 of the 34 were used to evaluate the photon models (Morris et al. 1993) and 7 experiments were used to evaluate the neutron models. Most of the early protracted irradiation publications included the graded mortality data from different exposure groups and we have used those data (from 34 experiments) previously. However, by the end of the 1950's, there became a general trend in radiation biology to publish only mathematical models of mortality and/or rates of recovery based on the estimated  $LD_{50}$  values associated with protocol variations--the valuable experimental dose-response data were not included in those publications. We did not use, or even consider, any of these studies on recovery models in our previous efforts involving model design and estimations of model parameters. Since our models and findings have been published since 1991, some questions have been raised, as described in the previous section, regarding our findings of radioresistance and slow repopulation of the hypothetical cell indicated to be "critical" to compensatory hematopoiesis by results of the MLE analysis. Because it is now desirable to obtain additional insight into the biological processes associated with acute mortality, those published  $LD_{50}$  values for protracted irradiations (i.e., results which we have not considered previously) are the only historical data now available to us for model validations and to address questions about whether a CFU-S based cell-kinetics model or a stromal-like model better standardizes the effects of protracted irradiation protocols. From an exhaustive search of the literature, we found a total of 44 additional studies, 38 of which are summarized in Table 6-1. The search found that 4 of the 38

additional studies had published some raw mortality data and for those studies we predicted group mortality in addition to estimates of the EPD based on the protracted  $LD_{50}$  values reported.

The lot, 72 experiments, 34 of which have been used to estimate constants in the model and 38 of which are used here to test those conclusions have result from an exhaustive literature review. Selection of the 38 experiments for model validation was based on: [1] dose protraction by rate or fractionation in mice or rats so that animal body size would not cause large uncertainties in marrow dose uniformity, [2] mortality within 30 days from the end of the radiation treatments (studies were used if a few animals died early from GI damage because it was assumed that those same animals would have died from marrow depression at a later time)--in contrast, studies were rejected if even a small number of animals died of marrow depression before the fractionated irradiation schedules were completed because the minimum effective dose could not be determined, [3] no more than 60 days between successive dose fractions, [4] equivalent handling of different phases of a particular experiment (e.g., uniform marrow doses and consistency in positioning the animals--confinement during irradiation was needed to be sure animals actually received the planned dosage, [5] adequate specifications of times or dose rates, and [6] the effort had to be reasonably successful at irradiating an adequate number of animals between the  $LD_{10}$  and the  $LD_{90}$ .

The 38 experiments used to validate the model (selected as described above) typically reported only  $LD_{50}$  values without reporting the actual dose-response data. Although these studies were not useful for estimation of model constants, they do provide independent tests of model utility. The 12 doses rate studies ranged from 0.08 to 474 r/min and the 26 fractionation studies contained treatment dose fractions ranging from 154 to 700 r given over periods ranging from hours to 8 weeks. Although the conversion of a protracted protocol to its prompt dose equivalence is dependent upon cell-lineage, that conversion for very simple fractionated protocols will sometimes produce numerically similar estimates of the EPD. In those instances, it is not clear which lineage better explains the molecular and cellular biology underlying acute mortality. In contrast, complex fractionation experiments and low dose-rate studies are sensitive to important lineage-specific effects and result in contrasting estimates for the EPD. As seen in the right-most column of Table 1-1, the results overwhelmingly indicate that a radioresistant, slowly repopulating cell is far more consistent with the cytological processes underlying acute mortality. Otherwise at least 50% of the cumulative probability distribution should be below the abscissa value of 1.0. Comparisons shown in Table 6-1 are based on the test hypothesis that EPD values computed from various protracted protocols should all converge to the EPD for the lineage of cells critically linked to hematopoietic survival.

A second comparison can be made based on how the EPDs for different protocols within the experiment compare with the EPD of the least protracted protocol of that group of experiments. That calculation is shown in Table 6-2 with the summary given in Table 6-3.

### 6.3 RESULTS.

The two models of marrow cell kinetics involve [a] cells that are "critical" to compensatory hematopoiesis with parameters estimated from MLE analysis of animal mortality data and [b] CFU-S type stem cells with parameters fitted from in vivo and in vitro cell-survival studies. As described in previous publications (Jones et al. 1993a, Jones et al. 1994a), both models seem to preform remarkably well according to the foundations of their evaluations. Clearly the point estimates and confidence intervals on estimated coefficients indicate that the two cellular models are distinct and do not simply provide different estimates for a common lineage.

For dose-response comparisons, (Jones 1981) found that normalization of the dose-related value to " $D/LD_{50}$ " (instead of using "D") standardized mortality data from practically any mortality experiment to a unitless numerical scale between 3/4 and 4/3. Later, from a larger data base, (Morris and Jones 1989) found that the  $LD_{90}/LD_{10}$  was characteristically less than 2.0 for continuous exposures. Clearly, the radiation mortality curve is very steep and errors in dosimetry or estimation of an equivalent prompt dose might be amplified by as much as 5- or 6-fold when dose is used to predict mortality or standardize observed mortality rates. Normally, a standard error (S.E.) of 8 to 10% for a dose-response related dependent variable is considered accurate, but for the dose-response behavior of hematopoietic mortality, it is essential to have the most accurate models possible for dosimetry-related decisions regarding patient care. Radiotherapists have long realized this behavior and since about 1975 have requested dosimetry precision to within 1 or 2 %, even for individual patients from a class of sick, diseased persons having intrinsic differences in biological response to a give dosage.

From the exercise of comparing grouping of the EPDs with the mean EPD of the group (assumes a normal distribution), the 38 protocols shown in Table 6-1—26 based on fractionation and 12 on variations in rate, indicate that about 70% of the protocols are significantly more consistent with an EPD based on a stromal-like cell. In contrast, about 15% of the protocols seem to be slightly more consistent with a CFU-S model of cell kinetics, but that group is associated with protocol variations where either cell kinetics model works reasonably well. Another 15% of the experiments seemed



consistent with either model. These comparisons were all based on the published  $LD_{50}$  level of response, but because we have found a probit model to adequately standardize mortality from continuous radiation schedules (Morris and Jones 1989), the conclusions should be similar at both high and low rates of mortality although the tails of the frequency distribution may be subject to a larger relative measure of randomness. The actual group data (in addition to being required for estimation of model coefficients) are also better for model validations because each  $LD_{50}$  is typically based on 5-10 treatment groups of test animals treated so that graded mortality ranged from 0 to 100%.

From the exercise of comparing the EPDs of a group of mortality experiments with the EPD of their least protracted member, column 5 of Table 6-3 indicates that about 70% of the experiments are more consistent with the stromal-like model with another 25% being similarly consistent with either model. Only 2/38 experiments were more consistent with the stem cell model but even those are not biologically or statistically consistent.

#### 6.4 DISCUSSION.

The exhaustive search for protracted irradiation studies on acute mortality and/or the  $LD_{50/30 \text{ day}}$  revealed an additional 44 experiments that were not used in any of our previous studies--which were based on 34 high quality protracted radiation experiments for which mortality data were published. Experimental studies were excluded from this validation effort for any of the following reasons: irradiations until death; times to death were more than 30 days after irradiation ended; and study protocols where equivalent treatment was not provided for different phases of the experiment, e.g., one study used X-rays,  $^{137}\text{Cs}$ , and 14 MeV neutrons but the animals were not irradiated or handled under equivalent conditions, and another experiment that used two biologically different dose rates interchangeably.

In addition to the four experiments that were excluded for these reasons, two other experiments are not included in Table 6-1. The first of those was a study by (Krebs and Brauer 1965) in which strain A female mice were given doses that were fractionated over 5, 10, 9-15, 15, 20, 30, 40, and 60 days. Because this was the only useable mortality data that we have been able to find for the A-strain mouse, we initially suspected that the A mouse might be somewhat abnormal with respect to recovery from radiation damage. However, the second problem study, conducted by (Krebs and Jones 1972) used the  $LAF_1$  mouse. Studies based on the  $LAF_1$  mouse were common to our data base and all of those other experiments were compatible with our dose protraction models and consistent with each other. Overall, we have not experienced problems that were presented by these two studies with any of the 72 other protracted studies.

Although we have been unable to identify any reasonable explanation as to why these two particular experiments are exceptional from our perspective, there are potential factors of significance: (Krebs and Jones 1972), although using the common LAF<sub>1</sub> mouse, [1] had problems with source positioning and delivery of the intended target doses, [2] used only 10 animals per group, [3] there was a major drift in radiosensitivity of the animal colony over the course of the study, and [4] they housed animals grouped so different treatment doses were in the same cage. [Later it became common knowledge that animals tend to live as a cage unit or die as a cage unit and it became common practice to have several different cages for each dose group of test animals.] (Krebs and Brauer 1965) provided no reasonable explanation as to why their study may have been exceptional, however they stated "The experimental results, however, indicated a pattern of recovery during fractionated exposure which was incompatible with previous studies of recovery."

## 6.5 CONCLUSIONS.

Until experimental studies found that cytokine-mediated processes, gap-junctions between cells, and supportive tissue structures were critical to toxicity-induced compensatory proliferation of a particular cellular lineage, there was widespread belief that epithelial cells, fibroblasts, and stromal tissues were probably not deterministically related to dose-response mechanisms. Commonly, those lineages were comprised of highly differentiated, slowly repopulating cells that had large  $D_0$  and  $n$  values from the fitted single target, multi-hit cell survival function. Although the microenvironment comprised of those radioresistant cells was required both in culture and in vivo for cell proliferation, it was consensus opinion that more rapidly proliferating, less terminally differentiated, and more radiosensitive cells determined the fate of dose-response.

Currently, two schools of opinion have emerged: one concerns itself predominantly with stem cells typically having  $D_0$  values roughly 2- to 5-fold lower than the  $D_0$  values of cytokine-producing stromal cells. Associated conclusions rationalize that these more sensitive and directly involved stem cells must be the gate-keepers of dose response. The other school of thought (which we have joined) is that because a wide array of animal experiments and human experiences have irrefutably shown that complete hematopoietic recovery can derive from a single cell that is commonly described as a "long-term repopulating cell (LTRC)," the functional integrity of the intact cellular microenvironment determines the fate of dose response.

Experimentally, the complex interdependent marrow lineages are difficult to control and it is even more difficult for the human brain to logically analyze the relative importance of the involved multiple simultaneous factors (even in other fields of study where data gaps are minimal). Studies on patterns of learning have found that human judgement is often wrong and scientists are only marginally better than non-scientists in this regard--in addition, scientists are sometime lead by ill-formed biases that do not affect the objectivity of their lay counterparts. (Note that such observations have been made simply on the basis of rationalization and would clearly not hold when powerful logic-enhancing tools such as multiple regression analysis or principles from artificial intelligence are used.)

For these and other reasons described in our previous publications, it seems to us that the best method of interpreting complex molecular and cellular processes is to use generic models of response-related mechanisms of action that have minimal dependence on assumptions and opinions. The output of that process can then be compared with the complex array of data and conclusions deriving from experimental hematology. From this logos, we--to date--have found no reason either to weaken or to discard our previous conclusion--that *"cells which regulate compensatory myelopoiesis correspond to marrow stromal or fibroblast cells with respect to radiosensitivity and doubling time."*

Table 6-1. Dose Protraction: Tier I summarizes studies in which high-dose rates (typically 4- to 8-fold higher than those used for bone marrow ablation in humans) were tested in combination with various fractionation patterns. Tier II summarizes continuous irradiations in which various degrees of dose protraction were obtained by lowering the dose rate.

Study	Protocol	# LD <sub>50</sub> s	LD <sub>50</sub> Range	(R/min)	Time (hr)	Exper-SE(%)
<b>I. Protracted mortality studies in which dose fractionations were used:</b>						
Hagan and Simmons (1956)	300 r + D <sub>2</sub> (t)	4	600 - 840 r	12	A/168-504	na
Mole (1956)	200 r + D <sub>2</sub> (t)	4	751 - 860 r	45	A/24-120	10
Spalding et al. (1961)	600 rad + D <sub>2</sub> (t)	7	766 - 1322 rad	9	A/21-547	20
Spalding et al. (1961)	205 rad + D <sub>2</sub> (t)	8	764 - 911 rad	9	A/24-216	9
Kohn and Kallman (1957)	315 r + D <sub>2</sub> (t)	5	570 - 873 r	30	A/72-504	13
Mole (1956)	600 r + D <sub>2</sub> (t)	5	751 - 1291 r	45	A/48-696	17
Mole (1956)	400 r + D <sub>2</sub> (t)	6	599 - 883 r	45	A/6-480	15
Spalding et al. (1961)	D <sub>1</sub> (t) + D <sub>2</sub> (t)	7	782 - 1078 rad	9	A/144	13
Tyler and Stearns (1964)	700 r + D <sub>2</sub> (t)	8	989 - 1232 r	50-60	A/1-24	12
Melville (1957)	D <sub>1/2</sub> (t) + D <sub>1/2</sub> (t)	4	519 - 893 r	70-120	A/48-192	62
Mole (1957)	6-60 F <sub>1</sub> (50 to 10 r)	21	750 - 2187 r	9,44	A/120-480	19
Kohn and Kallman (1957); C57BL	350 r + D <sub>2</sub> (t)	4	614 - 958 r	35	A/48-240	13
Kohn and Kallman (1957); C3H	350 r + D <sub>2</sub> (t)	4	623 - 948 r	35	A/48-192	18
Ainsworth et al. (1969); Table I	202 rad + D <sub>2</sub> (t)	9	315 - 466 rad	40	2-168	7
Melville (1957)	D <sub>1/2</sub> (t) + D <sub>1/2</sub> (t)	6	560 - 936 r	70-120	A/48-192	46
Patterson et al. (1952)	260 r + D <sub>2</sub> (t)	8	518 - 949 r	45	A/48-480	12
Kallman & Silini (1964)	457 r + D <sub>2</sub> (t)	24	683 - 1037 r	35	A/2-96	28
Dalrymple et al. (1963)	n x 300 r + D <sub>2</sub> (t)	5	880 - 1880 r	46	A/504-1344	25
Storer (1961)	D <sub>1</sub> (t) + D <sub>2</sub> (t)	10	674 - 1036 r	60	A/12-192	20
Brown et al. (1962)	2 - 18 Equal F <sub>1</sub>	24	955 - 1281 rad	55	6-120	18
Kallman & Silini (1964)	309 r + D <sub>2</sub> (t)	33	651 - 949 r	35	A/2-48	28
Kohn and Kallman (1957); A/Hc	350 r + D <sub>2</sub> (t)	4	619 - 990 r	35	A/48-192	30
Storer (1961)	D <sub>1</sub> (t) + D <sub>2</sub> (t)	8	675 - 937 r	60	A/48	20
Kallman and Silini (1964)	154 r + D <sub>2</sub> (t)	28	667 - 870 r	35	A/3-36	24
Kohn and Kallman (1957); Balb/c	350 r + D <sub>2</sub> (t)	5	552 - 882 r	35	A/48-336	20
Kohn and Kallman (1957); CAF <sub>1</sub>	350 r + D <sub>2</sub> (t)	6	673 - 1337 r	35	A/24-336	20
<b>II. Protracted mortality studies in which dose rate or radiation quality was varied:</b>						
Logie et al. (1960)	Dose Rates	5	908 - 2110 r	0.176-474	A-200	61
Thompson & Tourtellotte (1953)	Dose Rates	6	772 - 2760 r	0.08-42.2	A-276	20
Stearns & Tyler (1963)	Dose Rates	9	1012 - 1407 r	1-17	1-24	8
Corp & Neal (1959)	Acute, 25 H. & FO *	3	813 - 1340 rad	1.68, Var	A/25	10
Strike (1970)	Neut & γ	2	494 - 810 rad	20	A	8
Kallman (1962); C57BL	Dose Rates	4	624 - 823 r	2.19-18.4	0.6-6	20
Krebs (1975)	Dose Rates	5	905 - 1287 r	3.37-97.8	A-9	27
Dacquist and Blackburn (1960); rats	Dose Rates	2	498 - 684 r	3.30	A-3	28
Kallman (1962); Balb/c	Dose Rates	3	550 - 631 r	2.22-17.7	0.5-5	18
Hightower et al. (1968)	Dose Rates	4	392 - 424 rad	1-200	A-1	11
Hightower et al. (1968)	Dose Rates	4	412 - 457 rad	10-200	A-1	11
Dacquist and Blackburn (1960); mice	Dose Rates	2	650 - 743 r	3.30	A-3	24

\* "A" is used in column 6 to indicate an acute exposure usually lasting several minutes.

\* The fallout exposure involved variations in dose rates for 19 fractions given in 25 hours.

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results.

Ainsworth 1968 (Table I). Triga spectrum								
D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD (Stroma)	Σd/D
202	0.083	122	1.00	9	450	1.00	543	1.00
202	0.25	113	0.97	15	431	0.96	524	0.97
202	0.5	125	1.01	12	437	0.97	533	0.98
202	1	185	1.19	23	499	1.11	607	1.12
202	2	149	1.08	15	409	0.91	523	0.96
202	3	195	1.23	14	434	0.96	564	1.04
202	4	212	1.28	15	423	0.94	565	1.04
202	5	228	1.33	14	413	0.92	525	0.97
202	7	264	1.44	17	418	0.93	585	1.08
				24% = RMSD			7% = RMSD	6% = RMSD

mean EPD(Stem) = 435 ± 27 (C.V. = 0.062); mean EPD (Stroma) = 552 ± 30 (C.V. = 0.054)

Brown et al. 1962. 100 to 200 R-Strain mice per experiment given 250 kV @ 54.5 r/min

D <sub>1</sub> (r)	T (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD (Stem)	Σd/D	EPD (Stroma)	Σd/D
18 x (66.8)	41.1	1203	1.26	28	1047	1.12	811	0.95
18 x (63.5)	41.1	1143	1.19	17	988	1.06	770	0.90
18 x (62.5)	41.1	1125	1.17	18	970	1.04	750	0.87
18 x (67.4)	58.7	1214	1.27	19	1048	1.12	794	0.93
18 x (71.2)	83.7	1281	1.34	23	1102	1.18	817	0.95
4 x (256)	116	1025	1.07	15	988	1.06	837	0.98
4 x (289)	235	1157	1.21	36	1102	1.18	935	1.09
4 x (281)	235	1123	1.17	18	1068	1.14	905	1.05
4 x (284)	357	1137	1.19	38	1071	1.15	907	1.06
4 x (304)	475	1218	1.27	21	1140	1.22	967	1.13
2 x (479)	354	958	1.0	15	933	1.0	858	1.0
2 x (509)	353	1018	1.06	13	993	1.06	913	1.06
2 x (478)	354	955	1.00	15	930	1.00	855	1.00
2 x (488)	702	977	1.02	10	942	1.01	865	1.01
2 x (485)	714	970	1.01	22	935	1.00	859	1.00
2 x (532)	1072	1063	1.11	13	1016	1.09	934	1.09
2 x (534)	1072	1067	1.11	30	1020	1.09	937	1.09
2 x (498)	1434	995	1.04	16	938	1.01	862	1.00
2 x (491)	1434	982	1.03	15	925	0.99	850	0.99
2 x (532)	1792	1064	1.11	22	996	1.07	914	1.07
2 x (527)	2255	1054	1.10	15	973	1.04	892	1.04
2 x (528)	2866	1057	1.10	13	958	1.03	878	1.02
2 x (558)	4298	1116	1.16	31	974	1.04	892	1.04
2 x (579)	7198	1158	1.21	19	930	1.00	855	1.00
				17% = RMSD			10% = RMSD	6% = RMSD

mean EPD(Stem) = 999 ± 63 (C.V. = 0.063); mean EPD(Stroma) = 869 ± 54 (C.V. = 0.062)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protraction within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square deviation (RMSD) method was used to compare results. (Continued).

**Corp and Neal 1959.** Typically about 20 to 50 CBA mice per time-dose exposure group were irradiated with 250 kV @ 68.3 rad/min, for 25 hours at a fixed dose rate, and for 24 to 25 hours with a fallout-like decrease in dose rate

Rate <sub>1</sub> (rad/min)	Exposure	LD <sub>50</sub> (rad)	Σd/D	SE (rad)	EPD (Stem)	Σd/D	EPD (Stroma)	Σd/D
68.3	(12 min)	813	1.00	10	803	1.00	790	1.00
Variable	25 hr	1245	1.53	11	804	1.00	806	1.02
(~1)	25 hr	1340	1.65	9	755	0.94	801	1.01
				59% = RMSD	4% = RMSD		2% = RMSD	

mean EPD(Stem) = 787 ± 28 (C.V. = 0.036); mean EPD(Stroma) = 799 ± 8 (C.V. = 0.010)

**Dalrymple et al. 1963.** 300 to 1500 C57BJ6 mice per priming dose or control and telecobalt @ 46 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD (Stroma)	Σd/D
0	-	880	1.00	43	634	1.00	622	1.00
300	56	870	1.33	44	626	0.99	615	0.99
2 <sup>a</sup> x (300)	56	790	1.58	24	569	0.90	561	0.90
3 <sup>a</sup> x (300)	56	750	1.88	22	540	0.85	534	0.86
4 <sup>a</sup> x (300)	56	680	2.14	25	490	0.77	486	0.78
				79% = RMSD	15% = RMSD		14% = RMSD	

mean EPD(Stem) = 572 ± 60 (C.V. = 0.10); mean EPD(Stroma) = 564 ± 57 (C.V. = 0.10)

<sup>a</sup> 21 days between priming doses fractions; <sup>b</sup> based on 250 kVp

**Dacquist and Blackburn 1960.** Typically 20 to 35 Walter Reed-Bagg mice per time-dose exposure group were irradiated with 250 kVp @ 3 or 30 r/min

Rate <sub>1</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD (Stem)	Σd/D	EPD (Stroma)	Σd/D
30	(22)	650	1.00	42	632	1.00	618	1.00
3	(248)	743	1.14	51	594	0.94	568	0.92
				14% = RMSD	6% = RMSD		8% = RMSD	

mean EPD(Stem) = 613 ± 26 (C.V. = 0.042); mean EPD(Stroma) = 593 ± 35 (C.V. = 0.059)

**Dacquist and Blackburn 1960.** 15 Walter Reed-Cartworth Farm rats per time-dose exposure group were irradiated with 250 kVp @ 3 or 30 r/min

Rate <sub>1</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD (Stem)	Σd/D	EPD (Stroma)	Σd/D
30	(17)	498	1.00	54	484	1.00	479	1.00
3	(228)	684	1.37	58	548	1.13	526	1.10
				37% = RMSD	13% = RMSD		10% = RMSD	

mean EPD(Stem) = 516 ± 45 (C.V. = 0.087); mean EPD(Stroma) = 502 ± 33 (C.V. = 0.066)

**Hagan and Simmons 1956.** 6 or 7 groups of 10 or 20 Sprague Dawley rats and 200 kV @ 12 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD (Stem)	Σd/D	EPD (Stroma)	Σd/D
0	-	600	1.00	-	562	1.00	542	1.00
300	7	450	1.25	-	469	0.83	549	1.01
300	14	500	1.33	-	472	0.84	538	0.99
300	21	540	1.40	-	505	0.90	540	1.00
				33% = RMSD	15% = RMSD		1% = RMSD	

mean EPD(Stem) = 502 ± 35 (C.V. = 0.070); mean EPD(Stroma) = 542 ± 5 (C.V. = 0.009)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protraction within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square deviation (RMSD) method was used to compare results. (Continued).

**Hightower et al. 1968.** Typically about 24 to 48 mice per time-dose exposure group were irradiated with fission neutrons @ 1, 10, 100, and 200 rad/min

Rate <sub>i</sub> (r/min)	Exposure	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD <sup>b</sup> (Stroma)	Σd/
1	(41)	412	0.90	18	605	0.89	714	0.90
10	(44)	436	0.95	38	651	0.95	761	0.96
100	(4)	422	0.92	10	630	0.92	739	0.93
200	(2)	457	1.00	24	682	1.00	793	1.00
				- 8% = RMSD	8% = RMSD		7% = RMSD	

mean EPD(Stem) = 642 ± 33 (C.V. = 0.051); mean EPD(Stroma) = 751 ± 34 (C.V. = 0.045)

**Hightower et al. 1968.** Typically 24 to 48 ICR mice per time-dose exposure group were irradiated with fission neutrons @ 10, 100, and 200 rad/min

Rate <sub>i</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD <sup>b</sup> (Stroma)	Σd/
10	(41)	408	0.96	13	610	0.96	717	0.97
50	(8)	392	0.92	22	586	0.93	692	0.93
100	(4)	400	0.94	25	598	0.94	705	0.95
200	(2)	424	1.00	11	633	1.00	742	1.03
				- 6% = RMSD	6% = RMSD		5% = RMSD	

mean EPD(Stem) = 607 ± 20 (C.V. = 0.033); mean EPD(Stroma) = 714 ± 21 (C.V. = 0.029)

**Kallman 1962.** Typically 8 to 12 C57BL mice per time-dose exposure group were irradiated with 120 kVp @ 2.19, 4.84, 9.51, or 18.4 r/min

Rate <sub>i</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD <sup>b</sup> (Stroma)	Σd/
2.19	(376)	823	1.32	28	743	1.06	681	1.03
4.84	(148)	717	1.15	20	733	1.05	664	1.00
9.51	(71)	678	1.09	19	737	1.05	678	1.03
18.4	(34)	624	1.00	21	701	1.00	661	1.00
				21% = RMSD	5% = RMSD		2% = RMSD	

mean EPD(Stem) = 729 ± 19 (C.V. = 0.026); mean EPD(Stroma) = 671 ± 10 (C.V. = 0.015)

**Kallman 1962.** Typically 8 to 12 BALB/c mice per time-dose exposure group were irradiated with 2.22, 4.73, or 17.7 r/min

Rate <sub>i</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	Σd/D	SE (r)	EPD <sup>b</sup> (Stem)	Σd/D	EPD <sup>b</sup> (Stroma)	Σd/
2.22	(284)	631	1.15	15	574	0.93	542	0.93
4.73	(131)	618	1.12	24	631	1.02	581	0.99
17.7	(31)	550	1.00	18	617	1.00	585	1.00
				14% = RMSD	5% = RMSD		5% = RMSD	

mean EPD(Stem) = 607 ± 30 (C.V. = 0.049); mean EPD(Stroma) = 569 ± 24 (C.V. = 0.042)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

Kallman and Silini 1964. typically from 40 to 56 male C57BL/Ka mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	697	1.00	13	681	1.00	665	1.00
154	3	563	1.03	25	690	1.01	644	0.97
154	6	612	1.10	17	734	1.08	687	1.03
154	9	595	1.07	26	713	1.05	669	1.01
154	12	569	1.04	17	683	1.00	644	0.96
154	24	662	1.17	24	758	1.11	723	1.09
154	36	698	1.22	32	778	1.14	750	1.13
0	-	709	1.00	17	692	1.00	676	1.00
154	2	586	1.04	26	714	1.03	666	0.99
154	4	601	1.00	20	726	1.05	678	1.00
154	6	585	1.04	23	708	1.02	662	0.98
154	8	585	1.04	23	705	1.02	661	0.98
154	10	575	1.03	27	692	1.00	651	0.96
154	12	633	1.11	26	746	1.08	702	1.04
154	18	654	1.14	26	758	1.10	718	1.06
154	24	652	1.14	23	748	1.08	714	1.06
154	36	716	1.23	24	795	1.15	766	1.13
0	-	676	1.00	18	641	1.00	627	1.00
154	2	550	1.09	30	679	1.06	633	1.01
154	4	513	0.99	35	640	1.00	598	0.95
154	6	568	1.07	33	691	1.08	647	1.03
154	8	586	1.09	23	706	1.10	662	0.99
154	10	569	1.07	17	686	1.07	645	1.03
154	12	574	1.08	26	688	1.07	649	1.04
154	18	632	1.16	17	736	1.15	698	1.11
154	24	660	1.20	26	756	1.18	721	1.15
154	30	614	1.14	29	703	1.10	676	1.08
154	36	716	1.29	32	795	1.24	766	1.22

13% = RMSD

10% = RMSD

8% = RMSD

mean EPD(Stem) = 716 ± 40 (C.V. = 0.056); mean EPD(Stroma) = 678 ± 42 (C.V. = 0.062)



Table 6-2.

Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

Kallman and Silini 1964. typically from 40 to 56 male C57BL/Ka mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	0	692	1.00	17	676	1.00	660	1.00
309	6	459	1.11	19	737	1.09	676	1.02
309	12	406	1.03	24	675	1.00	622	0.94
309	24	470	1.15	40	717	1.06	666	1.01
309	36	487	1.15	21	713	1.05	669	1.01
309	48	583	1.29	31	786	1.16	743	1.13
0	0	615	1.00	18	636	1.00	623	1.00
309	3	390	1.14	23	675	1.06	618	0.99
309	6	401	1.15	21	681	1.07	624	1.00
309	9	402	1.16	28	676	1.06	622	1.00
309	12	365	1.10	43	635	1.00	585	0.94
309	24	472	1.27	29	719	1.13	668	1.07
0	0	725	1.00	22	708	1.00	691	1.00
309	2	404	0.99	44	689	0.97	631	0.91
309	4	472	1.08	32	753	1.06	691	1.00
309	6	447	1.04	22	725	1.02	666	0.96
309	8	461	1.06	26	736	1.04	676	0.98
309	10	400	0.98	33	673	0.95	619	0.90
309	12	480	1.08	22	747	1.06	688	1.00
309	18	524	1.15	33	780	1.10	721	1.04
309	24	528	1.15	32	773	1.09	718	1.04
0	0	659	1.00	20	643	1.00	630	1.00
309	2	440	1.14	28	724	1.13	664	1.05
309	4	454	1.16	22	736	1.14	674	1.07
309	6	460	1.17	30	738	1.15	677	1.07
309	8	514	1.25	23	787	1.22	724	1.15
309	10	422	1.10	25	694	1.08	639	1.01
309	12	482	1.20	30	749	1.16	690	1.10
309	18	546	1.30	34	801	1.25	741	1.18
309	24	622	1.41	40	865	1.35	803	1.27
309	30	574	1.34	29	808	1.26	753	1.20
309	36	588	1.36	31	811	1.26	760	1.21
309	48	640	1.43	28	842	1.31	795	1.26

21% = RMSD

15% = RMSD

11% = RMSD

mean EPD(Stem) =  $731 \pm 58$  (C.V. = 0.079); mean EPD(Stroma) =  $680 \pm 53$  (C.V. = 0.078)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued).

**Kallman and Silini 1964.** typically from 40 to 56 male C57BL/Ka mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	687	1.00	25	671	1.00	656	1.00
457	2	347	1.17	28	778	1.16	713	1.09
457	4	376	1.21	41	806	1.20	735	1.12
457	6	341	1.16	32	769	1.15	704	1.07
457	8	340	1.16	28	764	1.14	700	1.07
457	10	366	1.20	42	786	1.17	720	1.10
457	12	355	1.18	37	772	1.15	707	1.08
457	18	396	1.24	38	801	1.19	734	1.12
457	24	392	1.24	30	787	1.17	721	1.10
457	48	580	1.51	32	927	1.38	853	1.30
457	96	580	1.51	28	844	1.26	791	1.21
0	-	683	1.00	21	667	1.00	652	1.00
457	2	294	1.10	26	726	1.09	667	1.02
457	4	303	1.11	27	735	1.10	674	1.03
457	6	353	1.19	32	781	1.17	715	1.10
457	8	300	1.11	21	725	1.09	665	1.02
457	10	291	1.10	26	713	1.07	654	1.00
457	12	322	1.14	29	740	1.11	678	1.04
457	18	362	1.20	22	768	1.15	704	1.08
457	24	367	1.21	21	762	1.14	699	1.07
457	30	447	1.32	24	830	1.24	760	1.17
457	36	409	1.27	30	782	1.17	718	1.10
457	48	493	1.39	29	843	1.26	775	1.19
457	96	566	1.50	25	830	1.24	778	1.19
				27% = RMSD			19% = RMSD	13% = RMSD

mean EPD(Stem) =  $772 \pm 60$  (C.V. = 0.078); mean EPD(Stroma) =  $716 \pm 50$  (C.V. = 0.070)

**Kohn and Kallman 1957.** 803 FAC(I)F<sub>1</sub> rats were treated with 250 kVp @ 30 r/min in 6 experiments

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	570	1.00	7	555	1.00	546	1.00
315	3	378	1.22	14	549	0.99	566	1.04
315	7	464	1.37	16	510	0.92	595	1.09
315	14	502	1.43	16	494	0.89	571	1.05
315	21	558	1.53	13	543	0.98	587	1.08
				40% = RMSD			7% = RMSD	7% = RMSD

mean EPD(Stem) =  $530 \pm 27$  (C.V. = 0.051); EPD(Stroma) =  $573 \pm 19$  (C.V. = 0.033)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

**Kohn and Kallman 1957.** About 40 A/He mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

Kohn and Kohnen 1957. About 40 A/Nc lines per LD <sub>50</sub> determination were treated with 200 mg of 100% DDT per 100 g of dry weight of foliage.								
D <sub>1</sub>	T	LD <sub>50</sub>	d/D	SE	EPD	d/D	EPD	d/D
(r)	(day)	(r)		(r)	(Stem)		(Stroma)	
0	-	619	1.00	15	605	1.00	593	1.00
350	2	460	1.31	6	706	1.17	663	1.11
350	4	566	1.46	7	729	1.20	710	1.20
350	8	640	1.59	8	682	1.13	710	1.20
				47%=RMSD	17%=RMSD		18%=RMSD	
mean EPD(Stem) = 680 ± 54 (C.V. = 0.079); mean EPD(Stroma) = 669 ± 55 (C.V. = 0.082)								

**Kohn and Kallman 1957.** About 40 Balb/c mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	552	1.00	15	539	1.00	532	1.00
350	2	309	1.19	3	559	1.04	527	0.99
350	4	427	1.41	3	594	1.10	585	1.10
350	8	510	1.56	5	555	1.03	592	1.11
350	14	532	1.60	4	527	0.98	559	1.05
				47% = RMSD	6% = RMSD		8% = RMSD	
mean EPD(Stem) = 555 ± 25 (C.V. = 0.045); mean EPD(Stroma) = 559 ± 30 (C.V. = 0.054)								

**Kohn and Kallman 1957.** About 40 CAF<sub>1</sub> mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	673	1.00	10	657	1.00	643	1.00
350	1	413	1.15	6	702	1.07	648	1.01
350	2	479	1.23	4	724	1.10	680	1.06
350	4	586	1.39	4	749	1.14	728	1.13
350	8	687	1.54	5	728	1.11	753	1.17
350	14	677	1.53	5	668	1.02	691	1.07
				40%=RMSD	10%=RMSD		10%=RMSD	
mean EPD(Stem) = 705 ± 36 (C.V. = 0.051); mean EPD(Stroma) = 691 ± 44 (C.V. = 0.064)								

**Kohn and Kallman 1957.** About 40 C3H mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	623	1.00	18	608	1.00	597	1.00
350	2	453	1.29	5	699	1.15	656	1.10
350	4	535	1.42	6	699	1.15	682	1.14
350	8	598	1.52	6	641	1.05	672	1.13
				42%=RMSD			13%=RMSD	12%=RMSD
mean EPD(Stem) = 662 ± 45 (C.V. = 0.068); mean EPD(Stroma) = 652 ± 38 (C.V. = 0.058)								

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

**Kohn and Kallman 1957.** About 40 C57BL mice per LD<sub>50</sub> determination were treated with 250 kVp @ 35 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	614	1.00	13	600	1.00	589	1.00
350	2	469	1.33	7	715	1.19	671	1.14
350	4	576	1.51	5	655	1.09	674	1.14
350	10	608	1.56	7	622	1.04	659	1.12

48% = RMSD

12% = RMSD

13% = RMSD

mean EPD(Stem) = 648 ± 50 (C.V. = 0.077); mean EPD(Stroma) = 648 ± 40 (C.V. = 0.062)

**Krebs 1975.** Typically between 12 and 36 CF1 mice per time-dose exposure group were irradiated with <sup>60</sup>Co at 51.5, 26.7, 13.7, 6.75, or 3.37 r/min

Rate <sub>1</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD <sup>b</sup> (Stem)	d/D	EPD <sup>b</sup> (Stroma)	d/
51.5	(17)	905	1.00	15	654	1.00	642	1.00
26.7	(25)	932	1.03	15	660	1.01	639	1.00
13.7	(75)	1029	1.14	83	702	1.07	664	1.03
6.75	(162)	1095	1.21	27	697	1.07	651	1.01
3.37	(382)	1287	1.42	45	731	1.12	689	1.07

25% = RMSD

8% = RMSD

4% = RMSD

mean EPD(Stem) = 689 ± 32 (C.V. = 0.046); mean EPD(Stroma) = 657 ± 20 (C.V. = 0.030)

**Logie et al. 1960.** Sprague Dawley rats were irradiated with <sup>60</sup>Co @ 5 different dose rates

Rate <sub>1</sub> (r/min)	Exposure	LD <sub>50</sub> (r)	d/D	SE (r)	EPD <sup>b</sup> (Stem)	d/D	EPD <sup>b</sup> (Stroma)	d/
474	(1.9 min)	908	1.00	17	906	1.00	903	1.00
55	(19 min)	1044	1.15	61	1023	1.13	997	1.10
3.41	(6.2 hr)	1277	1.41	112	986	1.09	921	1.02
0.487	(2.7 day)	1885	2.08	80	718	0.79	1022	1.13
0.176	(8.3 day)	2110	2.32	59	367	0.41	959	1.06

88% = RMSD

32% = RMSD

9% = RMSD

mean EPD(Stem) = 800 ± 269 (C.V. = 0.34); mean EPD(Stroma) = 960 ± 50 (C.V. = 0.052)

**Melville et al. 1957.** 4 dose groups of 23 or 24 RF female mice were treated with 250 kVp x @ 100 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	560	1.00	28	555	1.00	552	1.00
2 x (345)	2	690	1.23	69	599	1.08	565	1.02
2 x (383)	4	765	1.37	38	593	1.07	576	1.04
2 x (438)	5	875	1.56	44	660	1.19	638	1.16
2 x (396)	6	791	1.41	143	541	0.97	552	1.00
2 x (468)	8	936	1.67	47	602	1.08	616	1.12

47% = RMSD

10% = RMSD

9% = RMSD

mean EPD (Stem) = 592 ± 42 (C.V. = 0.071); mean EPD(Stroma) = 583 ± 36 (C.V. = 0.062)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

Melville et al. 1957. 4 dose groups of 23 or 24 RF male mice were treated with 250 kVp x @ 100 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	519	1.00	52	515	1.00	512	1.00
2 x (380)	2	760	1.46	38	669	1.29	625	1.22
2 x (422)	5	844	1.62	193	628	1.22	613	1.20
2 x (447)	8	893	1.72	72	565	1.10	586	1.14
				61% = RMSD			22% = RMSD	19% = RMSD

mean EPD (Stem) = 594 ± 68 (C.V. = 0.11); mean EPD(Stroma) = 584 ± 51 (C.V. = 0.087)

Mole 1956. 40 to 45 CBA mice per split dose determination were treated with 240 or 250 kV @ 43 or 48.8 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
200	1	611	1.08	27	754	1.02	715	0.99
200	2	653	1.14	6	760	1.03	739	1.02
200	5	660	1.15	14	694	0.94	710	0.98
0	-	751	1.00	6	737	1.00	721	1.00
				13% = RMSD			4% = RMSD	2% = RMSD

mean EPD(Stem) = 736 ± 30 (C.V. = 0.041); mean EPD(Stroma) = 721 ± 13 (C.V. = 0.018)

Mole 1956. 37 to 75 CBA mice per split dose determination were treated with 240 or 250 kV @ 43 or 48.8 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
400	0.25	645	1.18	15	1014	1.17	932	1.10
400	0.5	671	1.21	21	1029	1.19	947	1.12
400	1	599	1.13	14	937	1.08	865	1.02
400	2	638	1.18	12	933	1.08	868	1.03
400	5	740	1.29	18	911	1.05	884	1.05
0	-	883	1.00	14	866	1.00	844	1.00
				20% = RMSD			13% = RMSD	8% = RMSD

mean EPD(Stem) = 948 ± 62 (C.V. = 0.065); mean EPD(Stroma) = 890 ± 41 (C.V. = 0.046)

Mole 1956. 35 to 49 CBA mice per split dose determination were treated with 240 or 250 kV @ 43 or 48.8 r/min

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
600	2	309	1.21	15	810	1.10	737	1.02
600	5	359	1.28	11	732	0.99	665	0.92
600	11	473	1.43	17	602	0.82	608	0.84
600	29	691	1.72	15	678	0.92	680	0.94
0	-	751	1.00	6	737	1.00	721	1.00
				45% = RMSD			11% = RMSD	9% = RMSD

mean EPD(Stem) = 713 ± 76 (C.V. = 0.11); mean EPD(Stroma) = 682 ± 51 (C.V. = 0.075)

Table 6-2.

Experiments listed in Table 6-1 were used to compare how the EPDs for different protraction within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

Mole 1957. CBA mice received daily dose received daily conditioning doses of 50, 25, 10, or 0 r 5X/week and a conventional LD<sub>50</sub> experiment was conducted at 0, 5, 11, or 18 days after the last priming treatment. Source was 240 kVp @ 44 or 9.5 r/min

D <sub>1</sub> (r)	T (day)	Age Index <sup>c</sup>	LD <sub>50</sub> (r)	d/D	SE (r)		EPD (Stem)	d/D		EPD (Stroma)	d/D
5 x (50) 0	1	541	1.05	13		662	0.90		656	0.91	
10 x (50)	0	2	467	1.26	34		609	0.81		631	0.86
10 x (50)	5	2	600	1.43	18		618	0.82		661	0.90
10 x (50)	11	3	695	1.56	7		685	0.91		706	0.96
15 x (50)	0	3	373	1.46	19		522	0.69		562	0.76
20 x (50)	0	4	239	1.61	27		393	0.52		478	0.65
10 x (25)	0	2	671	1.20	13		720	0.96		729	0.99
20 x (25)	0	4	622	1.46	13		672	0.89		695	0.94
20 x (25)	5	4	718	1.59	13		714	0.95		734	1.00
40 x (25)	0	8	574	1.93	22		625	0.78		653	0.84
40 x (25)	5	8	656	2.03	21		653	0.82		678	0.87
40 x (25)	18	10	701	2.09	77		687	0.86		683	0.88
60 x (25)	0	12	593	2.57	8		650	0.81		709	0.91
60 x (25)	5	12	660	2.65	19		658	0.82		686	0.88
60 x (25)	18	14	687	2.68	11		674	0.84		670	0.86
20 x (10)	0	4	769	1.26	36		775	1.03		774	1.05
50 x (10)	0	10	762	1.55	12		770	0.96		786	1.01
50 x (10)	11	11	807	1.60	37		815	1.02		828	1.06
0	-	0	750	1.00	na		736	1.00		720	1.00
0	-	2	768	1.00	6		753	1.00		737	1.00
0	-	10	815	1.00	8		799	1.00		780	1.00
87% = RMSD							19% = RMSD			14% = RMSD	

Ignoring age, mean EPD(Stem) = 676 ± 95 (C.V. = 0.14); mean EPD(Stroma) = 693 ± 77 (C.V. = 0.11)

<sup>c</sup> Mole found radiosensitivity varied with age. He ran control 3 different age controls and comparisons here are to the Mole's closest age group.

Patterson et al. 1952. 88 to 100 of A-Strain mice were exposed to split doses of 250 kV @ 45 r/min.

D <sub>1</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	518	1.00	6	509	1.00	504	1.00
260	2	493	0.95	14	402	0.79	394	0.78
260	10	676	1.31	12	422	0.83	462	0.92
260	10	780	1.51	na	524	1.02	559	1.10
260	20	799	1.54	8	529	1.03	538	1.07
5 x (123)	1	616	1.19	25	427	0.84	390	0.77
10 x (78.4)	1	784	1.51	19	379	0.74	361	0.72
21 x (45.2)	1	949	1.83	13	178	0.35	263	0.52
				48% = RMSD	29% = RMSD		25% = RMSD	

mean EPD(Stem) = 421 ± 109 (C.V. = 0.26); mean EPD(Stroma) = 434 ± 100 (C.V. = 0.23)

Table 6-2.

Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

Spalding et al. 1961. RF female mice 177 to 360 animals per LD<sub>50</sub> with <sup>60</sup>Co @ 9 r/min

D <sub>i</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
205	24	559	1.00	8	634	0.93	618	0.93
205	52	580	1.03	27	612	0.90	618	0.93
205	73	609	1.07	15	613	0.90	629	0.95
205	100	572	1.02	9	558	0.82	588	0.89
205	144	640	1.11	7	596	0.87	625	0.95
205	189	649	1.12	9	592	0.87	619	0.94
205	216	706	1.19	17	638	0.93	656	0.99
0	-	764	1.00	6	683	1.00	661	1.00
				10% = RMSD			12% = RMSD	7% = RMSD

mean EPD(Stem) = 616 ± 37 (C.V. = 0.060); mean EPD (Stroma) = 627 ± 23 (C.V. = 0.037)

Spalding et al. 1961. RF female mice 171 to 192 animals per LD<sub>50</sub> with <sup>60</sup>Co @ 9 r/min

D <sub>i</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
600	21	201	0.96	22	670	0.98	632	0.95
600	69	364	1.26	20	699	1.02	667	1.01
600	172	411	1.32	14	538	0.79	569	0.86
600	237	525	1.47	11	538	0.79	602	0.91
600	312	670	1.66	24	619	0.90	671	1.01
600	547	722	1.73	32	644	0.94	652	0.98
0	-	766	1.00	10	685	1.00	662	1.00
				48% = RMSD			13% = RMSD	7% = RMSD

mean EPD(Stem) = 628 ± 67 (C.V. = 0.11); mean EPD (Stroma) = 636 ± 38 (C.V. = 0.060)

Spalding et al. 1961. RF female mice to animals per LD<sub>50</sub> with <sup>60</sup>Co @ 9 r/min

D <sub>i</sub> (r)	T (hr)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
205	144	676	1.13	13	628	0.90	652	0.97
302	144	692	1.27	18	663	0.95	697	1.03
408	144	586	1.27	9	607	0.87	655	0.97
512	144	520	1.32	8	607	0.87	645	0.96
603	144	475	1.38	18	632	0.90	649	0.96
692	144	382	1.37	35	623	0.89	619	0.92
0	-	782	1.00	8	699	1.00	675	1.00
				30% = RMSD			11% = RMSD	5% = RMSD

mean EPD(Stem) = 637 ± 32 (C.V. = 0.050); mean EPD (Stroma) = 656 ± 24 (C.V. = 0.037)

Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

**Stearner and Tyler 1963.** About 72 LAF<sub>1</sub> mice per time-dose exposure group were irradiated with <sup>60</sup>Co for 9 different exposure times

Rate <sub>i</sub> (r/min)	Exposure (min)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD <sup>b</sup> (Stem)	d/D	EPD <sup>b</sup> (Stroma)	d/
(16.9)	60	1012	1.00	85	949	1.00	902	1.00
(11.6)	90	1046	1.03	102	955	1.01	897	0.99
(9.05)	120	1086	1.07	101	968	1.02	904	1.00
(6.14)	180	1106	1.09	108	942	0.99	878	0.97
(4.70)	240	1127	1.11	91	922	0.97	864	0.96
(3.36)	360	1209	1.19	131	931	0.98	882	0.98
(2.60)	480	1249	1.23	108	911	0.96	880	0.98
(1.41)	960	1354	1.34	125	811	0.85	862	0.96
(1.02)	1440	1407	1.39	123	753	0.79	855	0.95
				22% = RMSD			9% = RMSD	3% = RMSD

mean EPD(Stem) = 905 ± 73 (C.V. = 0.081); mean EPD(Stroma) = 880 ± 18 (C.V. = 0.020)

**Storer 1961.** 3 to 5 groups of 10 RF/J mice and 250 kVp @ 60 r/min

D <sub>i</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	674	1.00	21	665	1.00	655	1.00
200	0.5	504	1.04	26	671	1.01	628	0.96
200	1	579	1.16	26	726	1.09	690	1.05
200	2	622	1.22	75	734	1.10	715	1.09
400	1	429	1.23	10	775	1.17	714	1.09
400	2	464	1.28	26	767	1.15	714	1.09
400	4	636	1.54	12	854	1.28	818	1.24
600	2	231	1.23	20	737	1.10	675	1.03
600	4	235	1.24	10	656	0.99	597	0.91
600	8	339	1.39	17	592	0.89	585	0.89
				10% = RMSD			14% = RMSD	11% = RMSD

mean EPD(Stem) = 718 ± 74 (C.V. = 0.10); mean EPD(Stroma) = 679 ± 68 (C.V. = 0.10)

**Storer 1961.** 3 to 5 groups of 10 RF/J mice and 250 kVp @ 60 r/min

D <sub>i</sub> (r)	T (day)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	2	674	1.00	21	665	1.00	655	1.00
100	2	690	1.17	17	728	1.09	719	1.10
200	2	662	1.28	75	773	1.16	753	1.15
300	2	637	1.39	28	839	1.26	799	1.22
400	2	464	1.28	26	767	1.15	714	1.09
500	2	330	1.23	20	735	1.11	674	1.03
600	2	231	1.23	20	737	1.11	675	1.03
650	2	144	1.18	17	702	1.06	648	0.99
				26% = RMSD			15% = RMSD	11% = RMSD

mean EPD(Stem) = 743 ± 52 (C.V. = 0.070); mean EPD(Stroma) = 705 ± 52 (C.V. = 0.074)



Table 6-2. Experiments listed in Table 6-1 were used to compare how the EPDs for different protractions within the experiment compare with the EPD of the least protracted protocol of that group of experiments. The root-mean-square-deviation (RMSD) method was used to compare results. (Continued)

**Strike 1970.** Typically 24 to 56 Sprague-Dawley rats per time-dose exposure group were irradiated with fission neutrons or 250 kVp X rays @ 20 or 21 rad/min

Rate <sub>i</sub> (r/min)	Exposure	LD <sub>50</sub> (r)	d/D	SE (r)	EPD <sup>b</sup> (Stem)	d/D	EPD <sup>b</sup> (Stroma)	d/
21	(39)	810	1.00	23	779	1.00	745	1.00
20	(25)	494	0.61	12	505	0.65	637	0.86
			-39% = RMSD			35% = RMSD		14% = RMSD
mean EPD(Stem) = 642 ± 194 (C.V. = 0.30); mean EPD(Stroma) = 691 ± 76 (C.V. = 0.11)								

**Thompson and Tourtellotte 1953.** 60 to 254 CF-1 mice per LD<sub>50</sub> study were irradiated with <sup>60</sup>Co @ 6 different dose rates

Rate <sub>i</sub> (r/min)	Exposure	LD <sub>50</sub> (r)	d/D	SE (r)	EPD <sup>b</sup> (Stem)	d/D	EPD <sup>b</sup> (Stroma)	d/
42.2	(18 min)	772	1.00	9	752	1.00	740	1.00
14.9	(53 min)	785	1.02	19	731	0.97	708	0.96
4.0	(3.3 hr)	800	1.04	20	641	0.86	631	0.85
1.45	(12 hr)	1010	1.31	32	618	0.82	676	0.91
0.1	(12 day) 1658	2.15	167		162	0.22	559	0.76
0.08	(24 day) 2760	3.58	na		124	0.17	579	0.78
			127% = RMSD			52% = RMSD		17% = RMSD
mean EPD(Stem) = 505 ± 285 (C.V. = 0.56); mean EPD(Stroma) = 649 ± 72 (C.V. = 0.11)								

**Tyler and Stearner 1964.** 50 LAF<sub>1</sub> mice per dose group and <sup>60</sup>Co @ 50 to 60 r/min

D <sub>i</sub> (r)	T (min)	LD <sub>50</sub> (r)	d/D	SE (r)	EPD (Stem)	d/D	EPD (Stroma)	d/D
0	-	989	1.00	6	966	1.00	944	1.00
700	60	1063	1.07	14	1031	1.07	954	1.01
700	120	1123	1.14	10	1091	1.13	1003	1.06
700	240	1195	1.21	8	1162	1.20	1067	1.13
700	360	1178	1.19	9	1146	1.19	1052	1.11
700	480	1172	1.19	13	1136	1.18	1043	1.10
700	960	1232	1.25	29	1177	1.22	1079	1.14
700	1440	1194	1.21	32	1120	1.16	1026	1.09
			19% = RMSD			17% = RMSD		10% = RMSD
mean EPD(Stem) = 1104 ± 72 (C.V. = 0.065); mean EPD(Stroma) = 1021 ± 50 (C.V. = 0.049)								

Table 6-3. The experiments described in Table 6-1 were used to compare how the EPDs associated with hematopoietic stem and marrow stromal lineages for various LD<sub>50</sub> protraction protocols compare with the corresponding EPD for the least protracted protocol of that group.

Study:	RMSDD(%) (Treatment Dose)	RMSDD(%) (EPD-Stem)	RMSDD(%) (EPD-Stroma)	RMSDD(EPD-Stem)/RMSDD(EPD-Stroma)
Hagan and Simmons 1956	33	15	1	15
Logie et al. 1960	88	32	9	3.6
Thompson and Toutellotte 1953	127	52	17	3.1
Stearner and Tyler 1963	22	9	3	3.0
Kallman 1962	21	5	2	2.5
Strike 1970	(-39%)	35	14	2.5
Spalding et al. 1961	30	11	5	2.2
Corp and Neal 1959	59	4	2	2.0
Mole 1956	13	4	2	2.0
Krebs 1975	25	8	4	2.0
Spalding et al. 1961	48	13	7	1.9
Brown et al. 1962	17	10	6	1.7
Tyler and Stearner 1964	19	17	10	1.7
Spalding et al. 1961	10	12	7	1.7
Mole 1956	20	13	8	1.6
Kallman and Silini 1964	27	19	13	1.5
Kallman and Silini 1964	21	15	11	1.4
Storer 1961	26	15	11	1.4
Mole 1957	87	19	14	1.4
Dacquistor and Blackburn 1960	37	13	10	1.3
Storer 1961	10	14	11	1.3
Ainsworth 1968	24	7	6	1.2
Hightower et al. 1968	(-6%)	6	5	1.2
Kallman and Silini 1964	13	10	8	1.2
Mole 1956	45	11	9	1.2
Melville et al. 1957	61	22	19	1.2
Patterson et al. 1952	48	29	25	1.2
Dalrymple et al. 1963	79	15	14	1.1
Hightower et al. 1968	(-8%)	8	7	1.1
Kohn and Kallman 1957	42	13	12	1.1
Melville et al. 1957	47	10	9	1.1
Kallman 1962	14	5	5	1.0
Kohn and Kallman 1957	40	7	7	1.0
Kohn and Kallman 1957	40	10	10	1.0
Kohn and Kallman 1957	47	17	18	0.9
Kohn and Kallman 1957	48	12	13	0.9
Kohn and Kallman 1957	47	6	8	0.75
Dacquistor and Blackburn 1960	14	6	8	0.75

## SECTION 7

### MORPHALLAXIS FROM RADIATION BIOLOGY TO BENZENE TOXICITY

The recovery to normal tissue homeostasis in the model is not dependent upon the insult, either physical or chemical, that caused the injury. Instead, the recovery associated with repair of sublethal cellular injury and repopulation are formulated completely in terms of biologically related concepts involving populations of cells, length of the mitotic cycles, mitotic delay in  $G_2$ , etc. Thus, although the injury used to stimulate the recovery shown in Figure 7-1 was due to ionizing radiations, other insults such as chemical and/or surgical ablation of the marrow used to create similar injury may, in principle, be compensated for according to recovery aspects of marrow cell kinetics. Of course, insults that have a long biological half-life, activate different mechanisms, or are associated with toxicity to non-hematopoietic organs may not necessarily act in the manner shown.

Benzene is highly mobile inside the body and for simplicity may--like ionizing radiations--be expected to act primarily upon cells present in the body at the time of exposure. For example, (Rickert et al. 1979) found the benzene half-times in different organs of male Fischer-344 rats to be 48 minutes over the first 9 hours of exposure to 500 ppm by inhalation. A plot of amount expired in air was biphasic with  $t_{1/2}$  times of 42 minutes and 13.1 hours. The fraction retained with the longer half-time is less than 5% of the exposure, and one or two half times associated of 13.1 hours is still shorter than the typical cell cycle for most multipotent cells and their supportive stroma.

*Benzene-Induced Neoplasia in Animals:* Nine experiments comprised: 6 different routes of administration, rats and mice as test species, treatment times in the general intervals of 2, 4, 12, and 24 months, plus variations in biological endpoint, dose, and dose rate. Obviously, the data grid is much too sparse to permit estimation of numerical coefficients even if the appropriate functional form of a biologically based dose-response model were known.

*Acute Mortality from Benzene Toxicity:* Fifteen experiments reflected: 6 different routes of administration, 7 test species, and exposure times ranging from minutes to 7 hours. In some regards, this data grid is more sparse than the neoplasia data, and in addition these data provide nothing useful to view/model the effects of dose protraction.

*Cytotoxicity of CFC and CFU-S Cells is Often Linked to Benzene Toxicity:* Seven publications described a rather limited variety of measurements for: CFC and CFU-S cells, treated by inhalation and subcutaneous injection, at different total doses and treatment concentrations, for various periods of time, and a wide range of post-exposure assay times. Those data are summarized in Table 7-1. The benzene experiments currently available are inadequate for development of biologically-based models, except for drawing of some very fragmentary conclusions such as those listed in Table 7-2.

Compensatory repopulation by a particular cell is modeled by  $\lambda_{NN}MF_{NN}$ . The doubling time  $T_D$  associated with a particular surviving fraction can be estimated by  $T_D = \ln(2) / (\lambda_{NN}MF_{NN})$  and is shown in Figure 7-2 for a  $\lambda$  of  $0.00022 \text{ min}^{-1}$ . The vectors shown in Figure 7-2 are estimated doubling times from experimental data of (Uyeki et al. 1977) and (Cronkite et al. 1982,1985).

Benzene-induced hematopoietic toxicity is viewed in the broader context of the spectrum of exposures that: (a) are pancytotoxic and (b) induce compensatory hematopoiesis during or as a consequence of injury. Chlorambucil, chloramphenicol, chloroquine, cyclophosphamide, diethylamide, griseofulvin, ethylene oxide, ionizing radiations, lysergic acid, melphalan, methoxypsoralen, phenylbutazone, procarbazine, phosphorothioic acid triethylenetriamide, 7,12-dimethylbenz(a)anthracene, 2-acetylaminophenanthrene, N,N'-2,7-fluorenylenebisacetamide, N-2-fluorenylacetamide, 1-methyl-1-nitrosourea, and N-isopropyl- $\alpha$ -(2-methylhydrazine)- $p$ -toluamide hydrochloride have been associated with leukemia in humans or animals. Several publications have concluded that injury to both hematopoietic stem cells and their cellular/cytokine mediated environment can be important to acute mortality and leukemogenesis. A number of experimental studies have found that all marrow-derived lineages can be regenerated from only one surviving pluripotent stem cell; whereas, a stroma of strong functional integrity is required to support that regeneration from a single surviving stem cell. For additional insight into the relative importances of stem and stromal lineages, especially as potentially related to benzene toxicity see publications by (Metcalf 1985, Dorschkind 1990, Harigay et al. 1981, Gill et al. 1980, Lemischke et al. 1986, Abkowitz et al. 1988, Turhan et al. 1989, Irons 1979, Golde et al. 1980, Frash et al. 1976, Laskin et al. 1989, Roberts 1988).

(Cronkite 1961) concluded that any agent which produces marrow aplasia is a "putative leukemogen." Later, (Adamson and Seiber 1981) noted that "It is possible that a given proportion of individuals who develop bone marrow depression as a consequence of chemical exposure may ultimately develop ANLL (sic., acute non-lymphatic leukemia) regardless of which agent produced the marrow toxicity, and indeed

all of the chemicals which have been implicated as leukemogens can be myelosuppressive. Nevertheless, there are also chemicals which are potent depressants of bone marrow function but that have not been associated with human ANLL." (Harigaya et al. 1981) have proposed that the role of benzene may be more of a promoter by forcing the pluripotent stem cells (that have been exposed to leukemogenic initiating agents prior to benzene exposure) to undergo compensatory hematopoiesis. Because of existing data and simple, well-established dosimetry models, the quantitative considerations, as described here, have been limited to exposures involving ionizing radiations and the relevance to benzene toxicity is implied by analogy of molecular-, cellular-, and organ-based processes.

As illustrated in Figure 7-3 (a), our generic model of radiation-induced compensatory hematopoiesis has led to a strongly supported hypothesis that cell-to-cell contact and/or cytokine mediated processes between stromal and stem cells establish both the radiosensitivity and proliferation kinetics of the cells that are "critical" to hematopoietic recovery. Model evaluations described in this paper indicate that even though stem cell survival is necessary, the rate limiting considerations seem to be associated with a more radioresistant and more slowly repopulating "critical" cell that is consistent with characteristics measured for marrow stroma and CFU-F type lineages. Findings that have produced Figure 7-3 (a) seem to be remarkably consistent with the benzene toxicity model as described by (Laskin, MacEachern, and Snyder 1989) reproduced with permission in Figure 7-3 (b), and it would provide useful perspective if they chose to consider the morphallaxis from benzene to radiation biology.

Table 7-1. Summary of experimental data on CFU-S and CFC cells following treatments with benzene.

Test Time	<Dosing Schedule>			# F <sub>c</sub> 's	Route	Max. Conc.	Dose Rate	Dose	S(Femur)	Assay
	h/d	d/w	w							
Uyeki et al. (1977)										
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	45	1
CFC	8	3.5	1	3.5	Inhal.	4,680	10,700	37,600	13	0
CFU-S	8	3	1	3	Inhal.	4,680	10,700	32,200	41	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	39	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	50	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	40	1
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	74	4
CFC	8	1	1	1	Inhal.	4,680	10,700	10,700	82	7
Gill et al. (1980)										
CFU-S	6	5	1	5	Inhal.	4,000	6,890	34,400	39	1
CFU-S	6	5	4	20	Inhal.	4,000	6,890	138,000	47	1
CFU-S	6	5	6	30	Inhal.	4,000	6,980	207,000	27	1
Green et al. (1981)										
CFU-S	6	5	1	5	Inhal.	1	2	9	100	0
CFU-S	6	5	1	5	Inhal.	10	17	85	100	0
CFU-S	6	5	1	5	Inhal.	103	177	887	53	0
CFU-S	6	5	1	5	Inhal.	306	527	2,630	19	0
CFU-S	6	5	1	5	Inhal.	603	1,040	5,190	39	0
CFU-S	6	5	1	5	Inhal.	1,280	2,200	11,000	45	0
CFU-S	6	5	1	5	Inhal.	2,420	4,160	20,800	37	0
CFU-S	6	5	1	5	Inhal.	4,860	8,370	41,900	32	0
CFU-S	6	5	10	50	Inhal.	10	17	827	97	0
CFU-S	6	5	26	130	Inhal.	302	520	67,600	7.7	0
CFU-C	6	5	26	130	Inhal.	302	520	67,600	7.3	0
CFU-S	6	5	1	5	Inhal.	103	177	887	55	0
CFU-S	6	5	1	5	Inhal.	306	527	2,630	18	0
CFU-C	6	5	1	5	Inhal.	103	177	887	82	0
Cronkite et al. (1982)										
CFU-S	6	5	1	1	Inhal.	400	689	689	85	1
CFU-S	6	5	1	2	Inhal.	400	689	1,380	52	1
CFU-S	6	5	1	4	Inhal.	400	689	2,760	35	1
CFU-S	6	5	1	5	Inhal.	400	689	3,440	22	1
CFU-S	6	5	1.5	8	Inhal.	400	689	5,510	20	1
CFU-S	6	5	4	20	Inhal.	400	689	13,800	40	1
CFU-S	6	5	4	20	Inhal.	400	689	13,800	12	2
CFU-S	6	5	7	35	Inhal.	400	689	24,100	42	2
CFU-S	6	5	8	40	Inhal.	400	689	27,600	43	1
CFU-S	6	5	9.5	48	Inhal.	400	689	32,700	12	1
CFU-S	6	5	11	48	Inhal.	400	689	32,700	40	7
CFU-S	6	5	12	48	Inhal.	400	689	32,700	42	14

Table 7-1. Summary of experimental data on CFU-S and CFC cells following treatments with benzene. (Continued).

Cronkite et al. (1985)										
CFU-S	6	5	2	10	Inhal.	10	17	172	98	1
CFU-S	6	5	2	10	Inhal.	25	43	431	109	1
CFU-S	6	5	2	10	Inhal.	100	172	1,720	67	1
CFU-S	6	5	2	10	Inhal.	400	689	6,890	45	1
CFU-S	6	5	2	10	Inhal.	300	517	5,170	93	14
CFU-S	6	5	2	10	Inhal.	300	517	5,170	105	28
CFU-S	6	5	4	20	Inhal.	300	517	10,300	90	14
CFU-S	6	5	4	20	Inhal.	300	517	10,300	94	28
CFU-S	6	5	8	40	Inhal.	300	517	20,700	53	28
CFU-S	6	5	8	40	Inhal.	300	517	20,700	61	56
CFU-S	6	5	8	40	Inhal.	300	517	20,700	113	112
CFU-S	6	5	16	80	Inhal.	300	517	41,300	24	3
CFU-S	6	5	16	80	Inhal.	300	517	41,300	30	14
CFU-S	6	5	16	80	Inhal.	300	517	41,300	46	28
CFU-S	6	5	16	80	Inhal.	300	517	41,300	57	56
CFU-S	6	5	16	80	Inhal.	300	517	41,300	60	112
CFU-S	6	5	16	80	Inhal.	300	517	41,300	98	175
Cronkite et al. (1989)										
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	11	1
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	70	32
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	67	67
CFU-S	6	5	1	2	Inhal.	3,000	5,170	10,300	85	214
CFU-S	6	5	4	19	Inhal.	316	544	10,300	39	1
CFU-S	6	5	4	19	Inhal.	316	544	10,300	112	32
CFU-S	6	5	4	19	Inhal.	316	544	10,300	99	66
CFU-S	6	5	4	19	Inhal.	316	544	10,300	116	214
Tunek et al. (1981)										
CFU-C	-	6	1	6	Subcut.	0.7	1	4	94	1
CFU-C	-	6	1	6	Subcut.	3.5	4	21	56	1
CFU-C	-	6	1	6	Subcut.	18	18	108	60	1
CFU-C	-	6	1	6	Subcut.	88	88	528	33	1
CFU-C	-	6	1	6	Subcut.	440	440	2,640	5	1
CFU-C	-	1	1	1	Subcut.	440	440	440	60	1
CFU-C	-	2	1	2	Subcut.	440	440	880	26	1
CFU-C	-	3	1	3	Subcut.	440	440	1,320	18	1
CFU-C	-	4	1	4	Subcut.	440	440	1,760	4.3	1
CFU-C	-	5	1	5	Subcut.	440	440	2,200	8.7	1

Table 7-2. Summary of experimental results on benzene toxicity to hematopoietic cells.

Uyeki et al. (1977)	<p>Dose Response: If the dose rate is held constant, there seems to be only a weak dose response for exposures of 8 h/d because of the limited range of data. However, if one of the experimental points is corrected for the delay in assay time, then the dose response becomes more consistent with other studies.</p> <p>Doubling Time/Assay time: If dose and dose-rate are held constant, the time delay used in the assay appears to be very important. The doubling time seems to range from about 3.6 to 5.7 days for compensatory proliferation associated with a survival of about 40%.</p>
Gill et al. (1980)	<p>Dose Response: Only three points are available for evaluation of a dose response, but Gill's CFU-S cells could be twice as resistant as Uyeki's CFC cells.</p>
Green et al. (1981)	<p>Assays were made on the day that the dosing ended. These data contain both dose and dose rate variations in experimental design.</p> <p>Dose Response: Assays were conducted on day 0. If corrected to day 1 these results seem to be comparable to the data of Uyeki et al. Because of a wide range of doses, Green's data show a strong dose response.</p> <p>Dose Rate: A very strong effect of dose rate for 6 h/d protocol is demonstrated. Dose Rate may be more biologically significant than the number of days exposed (when the exposure is for 4 to 6 hours per day).</p>
Tunek et al. (1981)	<p>Route of Intake: These data are for subcutaneous injection--toxicity appears greater than for inhalation.</p> <p>Dose Response: Seems to have the same functional shape as data of Green.</p> <p>Dose Rate: Shapes of the response vs dose rate plots are similar to those of Green but the magnitudes are different. From the literature, we have found absorption coefficients ranging from 0.28 to 0.60 (median = 0.47) for inhalation of benzene. It seems that a rigorous analysis of absorbed fraction coupled with the dosing protocol differences used with inhalation studies are adequate to bring the Tunek data in line with the inhalation data.</p>
Cronkite et al. (1982)	<p>Dose Response: Seems very consistent with that of Green and Tunek but all doses were given at concentrations of 400 ppm. Data are given as day of assay and proliferation according to: (day, %): (1d, 12%), (7d, 40%), (14d, 42%). Therefore, the doubling time seems to range from about 2.6 to 5.2 days for repopulation from a survival of about 12%.</p>
Cronkite et al. (1985)	<p>Dose Response: Seems consistent with previous discussions, but dose rates are mostly for 300 ppm.</p> <p>Doubling Time: Seems to be very long, probably about 40 days--looks inconsistent with other studies.</p>
Cronkite et al. (1989)	<p>Doubling time: Seems to be about 5.8 days from a survival of 11%.</p>



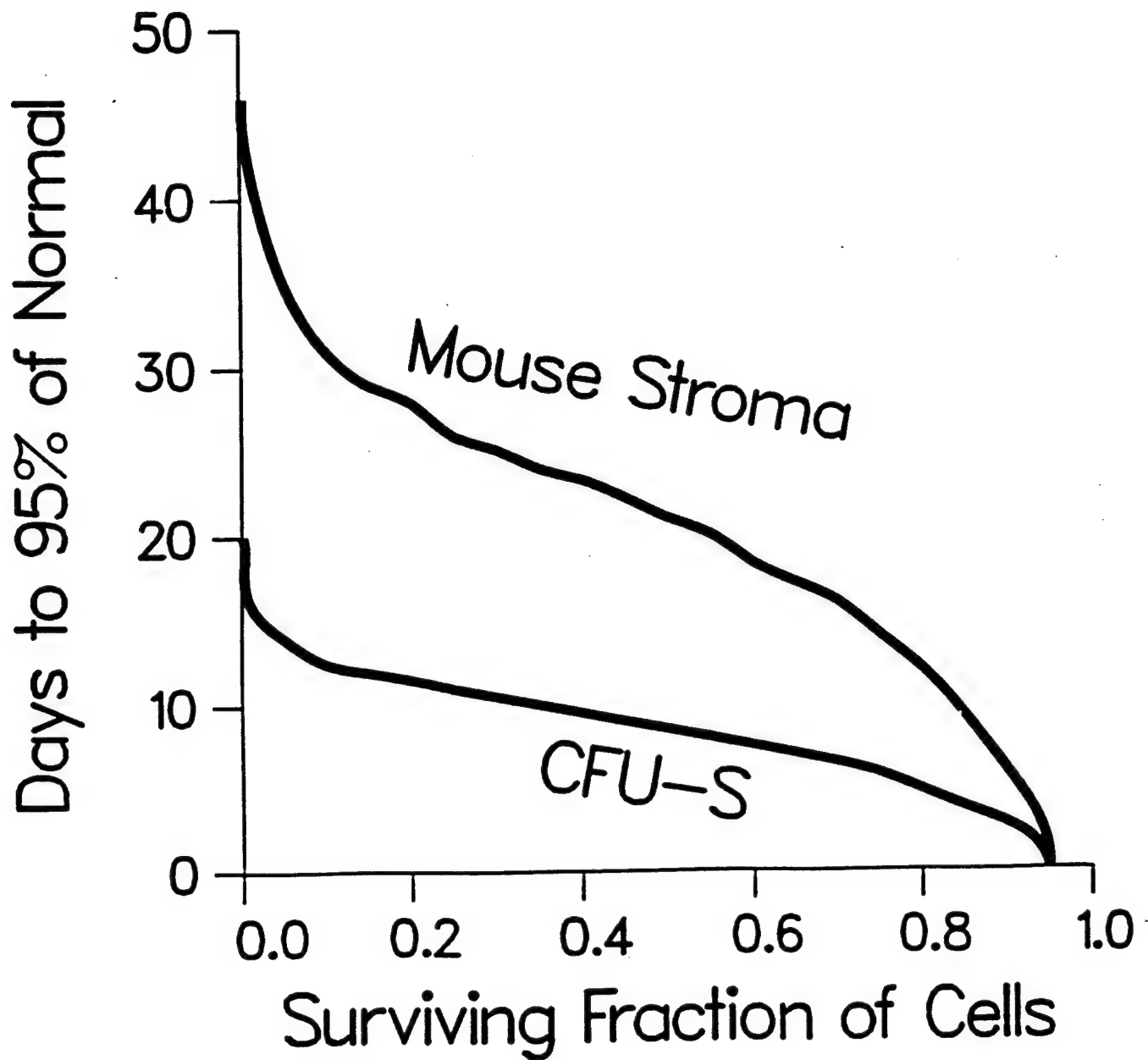


Figure 7-1. Time required to recover to 95% of normal vs cytopenia in a mouse.

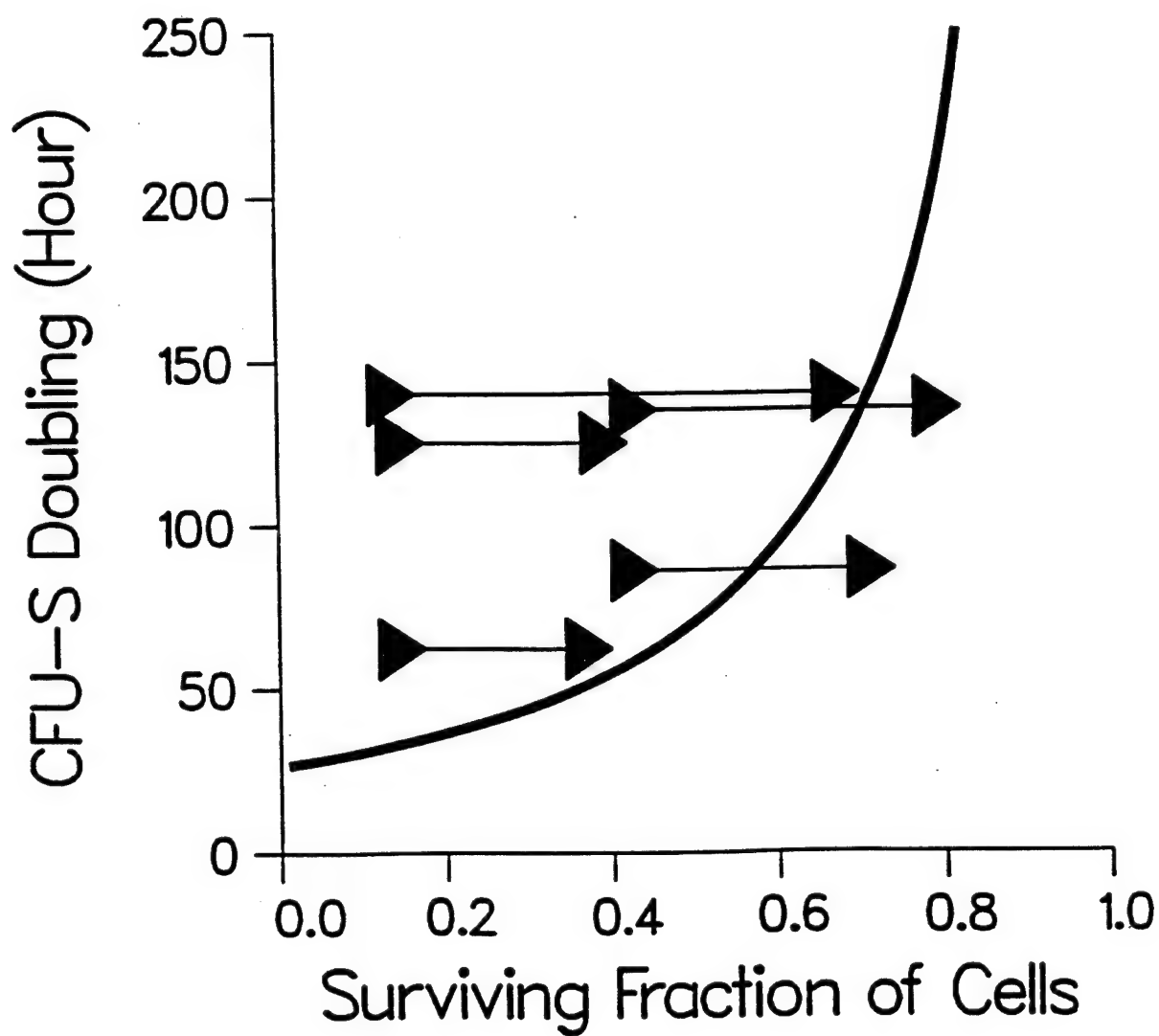


Figure 7-2. Doubling time for hematopoietic stem cells vs cytopenia.

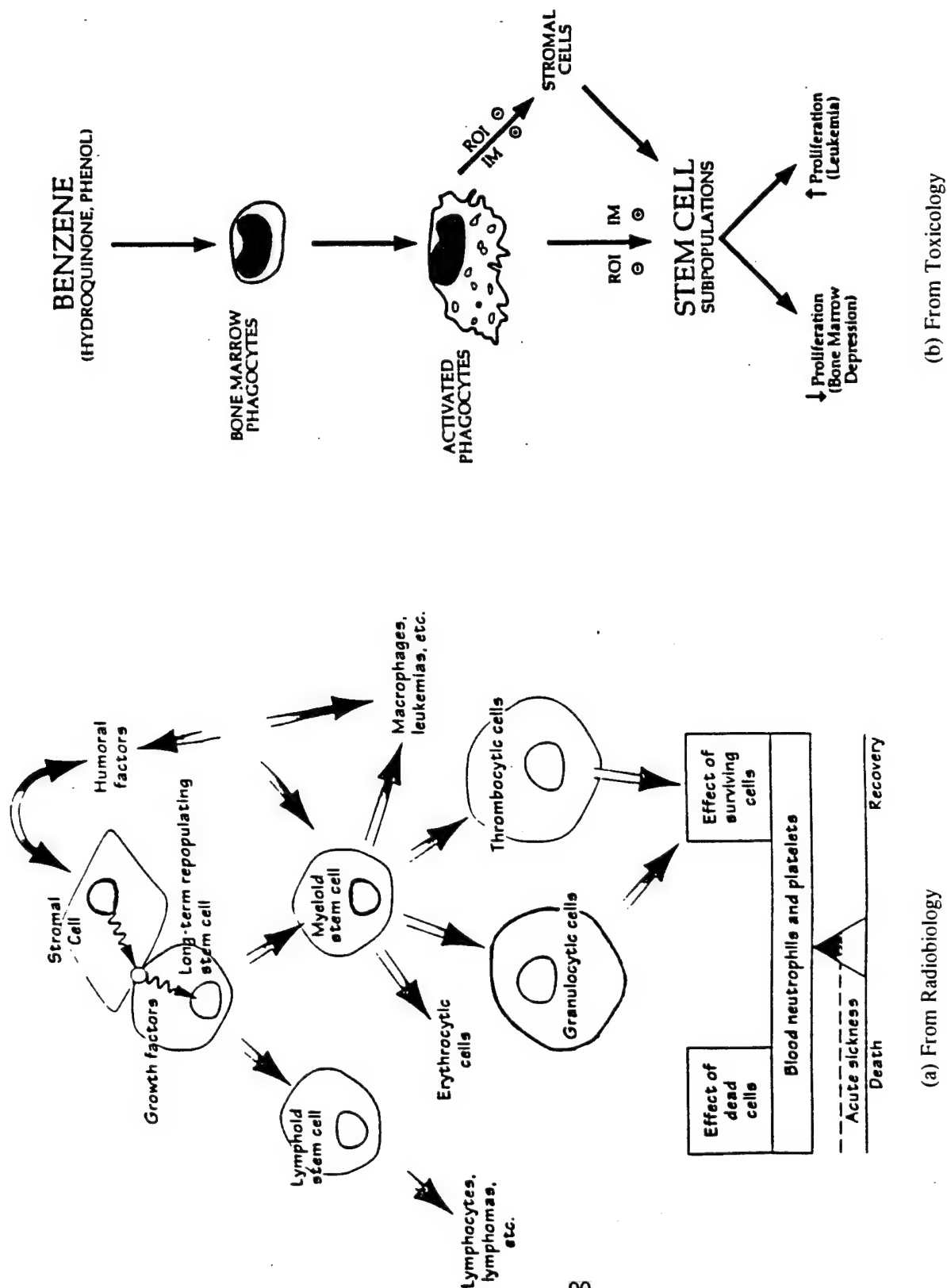


Figure 7-3. Stromal cell toxicity kinetics from (a) ionizing radiation and (b) benzene. Panel (b) is used by permission of Laskin et al. (1989). ROI = reactive oxygen intermediated; IM = immune modulators.

## SECTION 8

### STOCHASTIC CELL KINETICS MODEL

#### 8.1 INTRODUCTION.

Jones et al. (1991) described a mathematical model of the response of bone marrow to protracted doses of low l.e.t. radiation. The model is compartmental, describing the migration of cells in a critical population among "normal", "injured", and "killed" states as a function of time. Five rate coefficients appear in the model, controlling processes of cell injury (normal cells becoming injured), repair (injured cells becoming normal), death (normal and injured cells becoming killed by two separate processes), and proliferation (growth of the normal subpopulation by mitosis).

A connection between cell kinetics and hematopoietic death is also hypothesized, expressed through a definition of an "equivalent prompt dose". In short, the idea is as follows: Given a specified protracted exposure, there is some point in time -- at the end of the exposure or some earlier time -- at which the number of surviving cells, both normal and injured, is at a minimum. The equivalent prompt dose is defined as that unique instantaneous dose which would result in the same minimum number of surviving cells. Using this link between cell kinetics and mortality, Morris et al. (1991) used mortality data from several animal experiments to estimate the five rate coefficients in the cell kinetics model. The equations which define the model and a brief description of the terms appearing in them are given in the appendix.

The point of this note is to consider the effects of two assumptions tacitly made in that work. These are described below:

(A) In the development of the model, it is envisioned that there is some number of cells in the critical population ( $n_0$ ), and numbers of cells which are normal, injured, and killed ( $n_N$ ,  $n_I$ , and  $n_K$ ) which are functions of time. As presented, the model actually depends only upon the proportions  $n_N/n_0$ ,  $n_I/n_0$ , and  $n_K/n_0$ . The numbers of cells are tacitly assumed to be large, because these proportions are treated as continuous rather than discrete variables. For example, the model may stipulate a change of 3% in  $n_N/n_0$  over a certain period of time; if  $n_0$  is large and  $n_N$  is not very close to 0 this assumption will generally not present difficulties, but for  $n_N = 5$ , say, the smallest change physically possible would be 20%.

(B) The model and definition of equivalent prompt dose presented in these papers is deterministic rather than stochastic, in the following sense. Once a protracted exposure is stipulated, the minimum cell survival and time of that minimum are uniquely determined, and given the minimum level of surviving cells, the equivalent prompt dose is uniquely determined. Hence, this formulation assumes that two

hypothetically identical animals exposed to the same treatment would incur the same cellular damage, and identical equivalent prompt doses would be said to apply to both of them. Because, when fitting the model to data, we must reconcile cases in which two animals, which must be *treated* as identical, experience different endpoints, the usual approach to quantal dose-response modeling is then taken, where a specified equivalent prompt dose corresponds to a specific probability of death. However, the model does not allow for the possibility that some of this difference might be due to "identical" treatments having stochastically different real effects in "identical" animals.

In this note, we shall jointly examine the effects of these assumptions by considering a particular generalization of the cell kinetics model. This generalization, described in the next section, explicitly follows a finite number of cells (specified  $n_0$ ) through the cellular compartments, and defines the transitions as stochastic processes rather than by continuous deterministic rates of change. In Section 8.3, a modeling exercise based on a mouse experiment of Kaplan and Brown (1952) is described, and general conclusions are summarized in Section 8.4.

## 8.2 A STOCHASTIC GENERALIZATION OF THE DCKM.

The cell kinetics model described and analyzed by Jones et al. (1991) and Morris et al. (1991) is defined by a system of three differential equations, as explained in the appendix. The five basic processes by which cells migrate from one classification to another are each controlled by a rate coefficient. For example,  $\lambda_{NI}D'$  is the instantaneous rate at which normal cells are injured; this rate is proportional to the current dose rate ( $D'$ ), and the proportionality constant or "rate coefficient" ( $\lambda_{NI}$ ) controls the degree to which rate of cell damage increases with increasing dose rate. Hence in a short enough period of time,  $t_\Delta$ , the *number* of normal cells which become injured approaches:

$$\lambda_{NI}D't_\Delta n_N. \quad (8.1)$$

A negative term representing this quantity appears in the differential equation defining change in  $n_N$  (since these cells are leaving the normal state), and positively in the equation defining change in  $n_I$  (since they are entering the injured state). Similar expressions for the other four processes considered in the model - repair, direct killing, indirect killing, and proliferation - are described by similar expressions:

$$\begin{aligned} &\lambda_{IN}F_{IN}t_\Delta n_I, \\ &\lambda_{NK}D't_\Delta n_N, \\ &\lambda_{IK}D't_\Delta n_I, \\ &\lambda_{NN}F_{NN}t_\Delta n_N. \end{aligned} \quad (8.2)$$

Expressions corresponding to cellular repair and proliferation are somewhat more complicated due to nonlinear factors ( $F_{IN}$  and  $F_{NN}$  described in the appendix), but otherwise these rates of change operate in the same manner as with the simpler processes. In fact, the equations can be numerically solved in exactly this fashion; for relatively small time "steps", small changes in each of  $n_N$ ,  $n_I$ , and  $n_K$  are determined, and these quantities are updated for the calculation at the next time step. Since equivalent prompt dose is defined in terms of the minimum number of surviving cells over time:

$$\underline{n}_{NI} = \min_t (n_N(t) + n_I(t)), \quad (8.3)$$

this is a primary variable of interest. Again, this approach essentially assumes that  $n_N$  and  $n_I$  are smooth functions in time. Here, we shall refer to the cell kinetics model as presented by Jones et al. as the "Deterministics Cell Kinetics Model", or DCKM.

In this note, we shall examine how the model behaves when the numbers of cells in the three states are treated as discrete, and the movement of cells from state to state is governed by stochastic processes whose large-sample properties correspond to those of the DCKM model. An early reference to stochastic cell models which are in some ways similar to this is Hoel and Mitchell (1971). Returning to the process of normal cell injury discussed above, we now describe the "intensity" of the repair process by:

$$\lambda_{NI} D' n_N. \quad (8.4)$$

This means that in the absence of the other four cellular processes, the waiting time for the next repair event (i.e. return of a cell from the injured to the normal state) is an exponential random variable with mean  $(\lambda_{NI} D' n_N)^{-1}$ . Intensities for the other four processes are similarly defined, corresponding to how each of them would operate without the others.

The five cellular processes are modeled simultaneously as follows. At any given time, five random variables which follow independent exponential distributions are defined using the five intensities described above. Realizations (or values) are drawn from each of the five distributions, and the smallest of these values (i.e. the shortest waiting time) determines the kind of event which happens next. For example, if the realized waiting time for repair is the smallest of the five waiting times, the next event is a repair event resulting in one cell moving from the injured state to the normal state. None of the other four realized waiting times are operative; instead, five new realized waiting times are generated using the updated process intensities, and the next type of event is determined from the least of these. For large numbers of cells in each state, the number of cells moved by any one of the five processes in a short time interval is approximately a Poisson variate. The calculation proceeds as with the DCKM through the time steps which cover the exposure period, and the minimum value of surviving cells,  $\underline{n}_{NI}$ , is again calculated

for the time frame of interest. We shall refer to this modification as the "Stochastic Cell Kinetics Model", or SCKM.

Making this change in the model immediately implies two differences in how it must be used. First, a specific integer value of  $n_0$  must now be considered. (Recall that this is the initial total number of cells in the critical pool.) In the DCKM, this is not necessary. In fact, only the ratios  $n_N/N_0$ ,  $n_I/N_0$ , and  $n_K/n_0$  are really "identified" by the original model; changing  $n_0$  would only change  $n_N$ ,  $n_I$ , and  $n_K$  by the same proportion. But in the SCKM, because the specific value of  $n_0$  affects not only the mean but also the variance of the random processes in the generalized model, it must be considered. (Note that, for a given exposure scenario, if  $n_0$  is large enough, the results would be essentially the same for both DCKM and SCKM; variation in numbers of cells in each state, relative to their means, would essentially disappear, as demonstrated in the calculations of Section 8.2). One question of interest here is *how* large  $n_0$  must be for the simpler model to be adequate.)

Second, because the increments in numbers of cells representing each process are now random variables, the minimum number of surviving cells will also be random. That is, evaluating the model twice for the same exposure scenario will in general *not* lead to the same minimum number of surviving cells. Our interest will be in evaluating the SCKM a number of times for each exposure scenario of interest, to investigate the resulting *distribution* of  $\underline{n}_N$  values, and whether these can be used to produce better predictions of animal mortality than are generated by the DCKM.

We wrote a FORTRAN computer program which simulates the movement of a specified integral number of cells among the three states, as required by the SCKM, using exponential times-to-event as described above. This program was used in the calculations described in the following section.

### 8.3 KAPLAN AND BROWN EXPERIMENT.

In order to examine whether the SCKM provides a better fit to mortality data than the DCKM, we compared them using an experimental design and data from a mouse experiment reported by Kaplan and Brown (1952). In this work, male and female C57BL mice were exposed to 120 kVp x-rays at the rate of 0.28 Gray per minute to marrow. Thirty-five experimental groups, each containing between 27 and 91 animals, were exposed at total doses ranging from 2.52 to 10.07 Gray to marrow, the dose being delivered in from one to eight fractions. Total time of treatment (from the beginning of the first fraction to the end of the last) ranged from 9 minutes to 48 days, and the number of animals dying in each group with 30 days after exposure ended was reported. Details of exposures and survival of each group are given in Table 8-1.

The rate coefficient values used in this study were based on those reported as "estimate (a)" in Morris et al. (1991), which were derived from mortality data for mice exposed to 250 kVp x-rays. These coefficients were adjusted to account for the difference in energy spectra between 250 kVp x-rays and 120 kVp x-rays (used by Kaplan and Brown), as described in Morris et al. (1993); the adjusted estimates, and their units, are:

$$\begin{aligned}\lambda_{NI} &= 0.00585 \text{ cGy}^{-1} \\ \lambda_{IN} &= 0.0162 \text{ min}^{-1} \\ \lambda_{NK} &= 0.00396 \text{ cGy}^{-1} \\ \lambda_{IK} &= 0.00948 \text{ cGy}^{-1} \\ \lambda_{NN} &= 0.0000851 \text{ cGy}^{-1}\end{aligned}\tag{8.5}$$

We did not attempt to "fine tune" these values more closely for the Kaplan data set.

Using the DCKM with these coefficient values, values of  $\underline{n}_{NI}/n_0$  were calculated for each of the 35 experimental groups. Figure 8.1, panel a, displays how these minimum (over time) surviving cell fractions vary with the observed animal survival in each experimental group. The remaining two parameters which must be fitted to predict animal survival/mortality from the DCKM are the equivalent prompt dose-response characteristics -- in this case the  $LD_{50}$  and inverse slope for the probit model. For the Kaplan data set, the maximum likelihood estimates of these are 4.13 Gray and 0.83 Gray, respectively. The corresponding fitted probit model produces estimated mortality fractions in each group which are contrasted to their experimental counterparts in Figure 8.1, panel b.

As noted above, the concepts of cell survival and equivalent prompt dose are not so clear in the SCKM, since identical exposures result in different results. As a first comparison to the DCKM, we eliminated this variability by simulating the cell kinetics for each of the 35 treatments a large number of times, and considering the average value of  $\underline{n}_{NI}$  obtained in these repeated simulations. Figures 8-2, panels a-d, display a comparison of these average cell survival numbers to observed animal survival for  $n_0 = 10, 100, 1,000,$  and  $10,000,$  respectively. We then used these average values of  $\underline{n}_{NI}$  as we would with the DCKM, i.e. by calculating equivalent prompt doses, fitting a probit model relating these to animal mortality, and calculating the modeled animal mortality fraction for each group. The estimates of group mortality obtained in this way are compared to their experimental counterparts in Figures 8-3, panels a-d, for the four values of  $n_0$  given above.

Visual comparison of panels a and b of Figure 8-1 generated using the DCKM, to Figures 8-2, panels a-d, and 8-3, panels a-d, does not indicate a substantial difference in behavior between the two models, except possibly for the SCKM with  $n_0 = 10$ . However, in order to make the comparison more objective, two



indices were computed to quantify the differences in observed and predicted animal mortality proportions displayed in Figures 8-1, panel b, and 8-3:

$$S_1 = \frac{1}{35} \sum_{i=1}^{35} (m_i - o_i)^2 \quad (8.6)$$

$$S_2 = \frac{1}{35} \sum_{i=1}^{35} \frac{(m_i - o_i)^2}{\frac{1}{n_{i,0}} m_i (1 - m_i)} \quad (8.7)$$

where  $o_i$  and  $m_i$  are the observed and modeled proportions of mortality for group  $i$ , and  $n_{i,0}$  is the number of animals in the  $i$ th group of the (observed) experiment. These indices are simply the average squared difference between observed and fitted values, and a similar average in which each difference has been "normalized" by the standard deviation suggested by the binomial distribution; in either case, larger values correspond to less agreement between observed and modeled values. The following table displays these indices calculated for the SCKM with  $n_0 = 10, 100, 1,000$ , and  $10,000$ , and the DCKM:

model	$n_0$	$S_1$	$S_2$
SCKM	10	0.13125	2.48875
SCKM	100	0.11082	2.16834
SCKM	1,000	0.10966	2.13579
SCKM	10,000	0.10963	2.13103
DCKM	-	0.10932	2.12579

These results verify the impression one gets from looking at Figures 8-1, panel b, and 8-3; using *average* values of  $\underline{n}_{NI}$  derived under the SCKM in the same way as the *unique* values of  $\underline{n}_{NI}$  are used under the DCKM does not improve the mortality predictions. Results for  $n_0 = 10,000$  are very nearly the same as those for the DCKM (as should be expected since the DCKM is, in a sense, a "large sample limit" of the SCKM), and for smaller values of  $n_0$  the performance of the SCKM is worse. Figures 8-4, panels a-d, further demonstrate this by showing that the modeled surviving cell fractions under the DCKM are quite close to their counterparts under the SCKM.

This finding should not be surprising, because in taking an "average" value of  $\underline{n}_{NI}$ , we ignore what is potentially the greatest benefit to stochastic cell kinetics modeling, i.e. the *variability* of the outcome associated with given exposure condition. The standard deviation of  $\underline{n}_{NI}$  is plotted against average  $\underline{n}_{NI}$  in Figures 8-5, panels a-d, for  $n_0$  of 10, 100, 1,000, and 10,000, respectively. The first characteristic of variability apparent in these graphs is that variability increases with the mean. In fact, the variance of  $\underline{n}_{NI}$

(square of the standard deviation) could be modeled approximately as the mean or average. (This property is a characteristic of the Poisson distribution. Although  $\underline{n}_{NI}$  is not a Poisson variate under the SCKM, related values in simpler compartmental models would have this distribution. The few points which appear below the others in these plots, i.e. have smaller variability, given their average, than the general pattern would indicate, are associated with the longest experimental treatments -- groups 33 through 35 in Table 8-1.) The second characteristic of variability, related to the first, is that the standard deviation, expressed as a function of the average, diminishes as  $n_0$  increases. (This is why, as noted in the graphs, more simulations were performed for small values of  $n_0$  than for large ones. This was done to improve the estimates of the standard deviations relative to the means.) This is an intuitive explanation of why the SCKM "approaches" the DCKM as  $n_0$  increases; for large enough  $n_0$ , variability in  $\underline{n}_{NI}$  for a given exposure schedule becomes negligible compared to variability between average values of  $\underline{n}_{NI}$  for different exposure schedules.

A second, and perhaps more natural, way to associate the SCKM with animal mortality is to combine this variability of  $\underline{n}_{NI}$  with the concept that a "critical number" of cells must survive to enable the animal to survive. We denote this number (whatever its value) by  $n_{crit}$ , and say that any animal for which  $\underline{n}_{NI} \geq n_{crit}$  survives, while any animal for which  $\underline{n}_{NI} < n_{crit}$  dies. This eliminates dependence on the probit model, or for that matter on definition of an equivalent prompt dose, and is a generalization of the "tissue rescuing unit" concept considered by Hendry and Roberts(1971) and others. In this case, the "additional parameters" which must be fitted are  $n_0$  and  $n_{crit}$ , i.e. what are values for these two quantities which produce the best agreement with the observed mortality data?

In order to answer this question, we "simulated" 100 mice under each of the 35 experimental conditions (i.e. calculated 100 independent values of  $\underline{n}_{NI}$  corresponding to each treatment), using different values of  $n_0$ . We then calculated "simulated" proportions of animal mortality corresponding to different values of  $n_{crit}$ . Simulated and observed mortality proportions were compared using the index:

$$S_3 = \sum_{i=1}^{35} \frac{(m_i - o_i)^2}{\left( \frac{1}{n_{i,0}} + \frac{1}{n_{i,m}} \right) p_i (1-p_i)} \quad (8.8)$$

where  $o_i$  and  $m_i$  are the observed and simulated proportions of mortality for group  $i$ ,  $p_i$  is the "pooled" proportion of mortality using the both the observed and simulated data for group  $i$ ,  $n_{i,0}$  is the number of animals in the  $i$ th group of the (observed) experiment, and  $n_{i,m} = 100$  is the corresponding number in the simulation. Under a null hypothesis that the SCKM and animal mortality rule are correct, this statistic would approximately follow a chi-squared distribution. However, here we prefer to interpret it simply as a measure of agreement between observed and simulated data.

The parameter values which minimized  $S_3$  in this numerical experiment were somewhat smaller than we might have anticipated:  $n_0 = 12$  and  $n_{crit} = 1$ . Using these parameter values, we ran a much larger simulated experiment (10,000 simulated mice per group) to precisely predict the fraction of animal mortality which would be expected in each group. These predicted mortality fractions are plotted against their observed counterparts in Figure 8-6, which can be compared to the corresponding relationship for the DCKM in Figure 8-1, panel b. As with our first attempt, it seems clear from the graphs that this approach does not lead to an improvement (over the DCKM) in predicted mortality. To complete this evaluation, we recalculated  $S_1$  and  $S_2$ , as defined above, for this set of predictions and obtained values of 0.43043 and 8.36597, respectively, both worse than all other values of these indices reported above.

#### 8.4 DISCUSSION AND SUMMARY.

Variability of values of  $\underline{n}_{NI}$  generated by the SCKM generally behaves as we might expect; when median of the distribution is relatively small, the relative spread of the distribution is relatively great. This is common in models of discrete stochastic processes, for example simple Poisson processes. It seems unlikely that the internal variability of  $\underline{n}_{NI}$  induced by the SCKM will correspond to important variation in the probability of mortality unless the population of critical cells is smaller than is currently supposed. Clearly the current exercise is quite limited; we have considered only one mortality experiment in which one species of mouse was exposed to x-irradiation at a single rate. Further, we have adopted the five cell kinetics parameter values as estimated under the DCKM in a previous study, and have not tried to adjust these to improve performance of either the DCKM or SCKM here. However, within these constraints, it seems unlikely that the SCKM will offer significantly better predictions of animal mortality than the DCKM.

Perhaps the greater potential for usefulness of stochastic cell kinetics modeling lies in phenomena which (1) are initiated by a very small number of cells, and/or (2) require very long periods of time to exhibit the condition of interest -- e.g. have smaller process intensities than those used here. These are the general conditions under which the DCKM and SCKM should be expected to most dramatically differ, and the first condition describes where the "continuous" formulation of the DCKM is most questionable. The first condition would be typical, for example, of leukemogenesis, which may begin with a single cell. The second might be typical of the cellular kinetics following exposures to very low dose rates over very long time periods. It has been argued that in animal studies such exposures have been observed to "break down" the hematopoietic system's ability to regenerate, a phenomenon which is not predicted by the DCKM formulation.

The DCKM has been shown to have excellent predictive capability in modeling radiation-induced

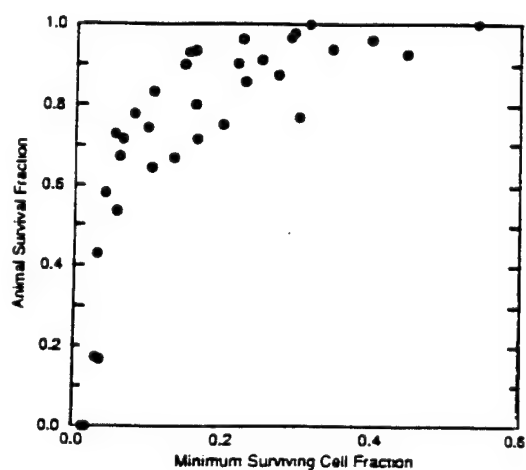
mortality for exposures given over time periods of up to several weeks; Morris et al. (1991 & 1993). Despite the fact that the SCKM extension is considerably more difficult to deal with computationally, it has not been shown to lead to improved predictions of mortality in this limited exercise. However, both approaches to cell kinetics modeling would seem to hold potential in other applications, and the SCKM in particular should be considered as an alternative to deterministic modeling when interest centers on slow processes involving a small pool of cells.

Table 8-1. Summary of Exposures and Data from Kaplan and Brown (1952) Experiment<sup>1</sup>.

group	number of fractions	length of fractions (minutes)	time between fractions (days)	animals	deaths
1	1	9.13	-	27	1
2	1	10.84	-	31	2
3	1	12.90	-	42	7
4	1	15.33	-	42	12
5	1	18.27	-	84	70
6	2	4.57	1	31	1
7	2	5.42	1	31	3
8	2	6.45	1	29	2
9	2	7.67	1	39	10
10	2	9.13	1	71	33
11	2	10.86	1	81	67
12	2	12.90	1	63	63
13	4	2.71	1	39	9
14	4	3.23	1	42	6
15	4	3.83	1	35	7
16	4	4.57	1	56	20
17	4	5.43	1	70	23
18	4	6.45	1	91	52
19	4	7.67	1	60	60
20	8	1.61	1	33	2
21	8	1.92	1	32	4
22	8	2.28	1	36	9
23	8	2.71	1	42	14
24	8	3.23	1	54	12
25	8	3.83	1	50	21
26	8	4.56	1	56	56
27	4	3.83	4	51	0
28	4	5.43	4	49	14
29	4	7.67	4	55	15
30	4	3.83	8	54	4
31	4	5.43	8	48	1
32	4	7.67	8	60	6
33	4	3.83	16	51	0
34	4	5.43	16	52	2
35	4	7.67	16	57	5

1: Animals were C57BL mice; radiation used was 120 kVp x-ray administered at the rate of 0.28 Gray per minute to marrow.

(a) Cell Survival



(b) Mortality

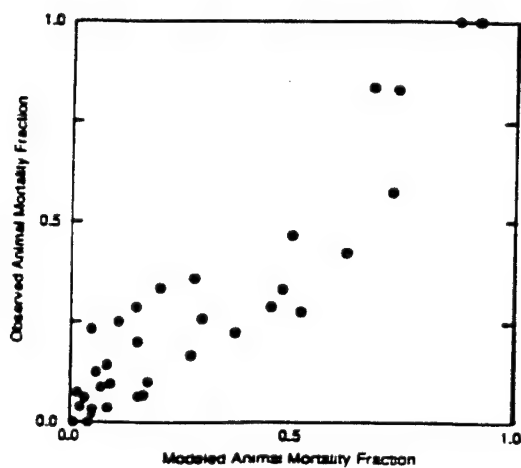


Figure 8-1. Deterministic Cell Kinetics Model: (a) Mean cell survival vs survival from the DCKM model, and (b) modeled vs observed mortality for the DCKM model.

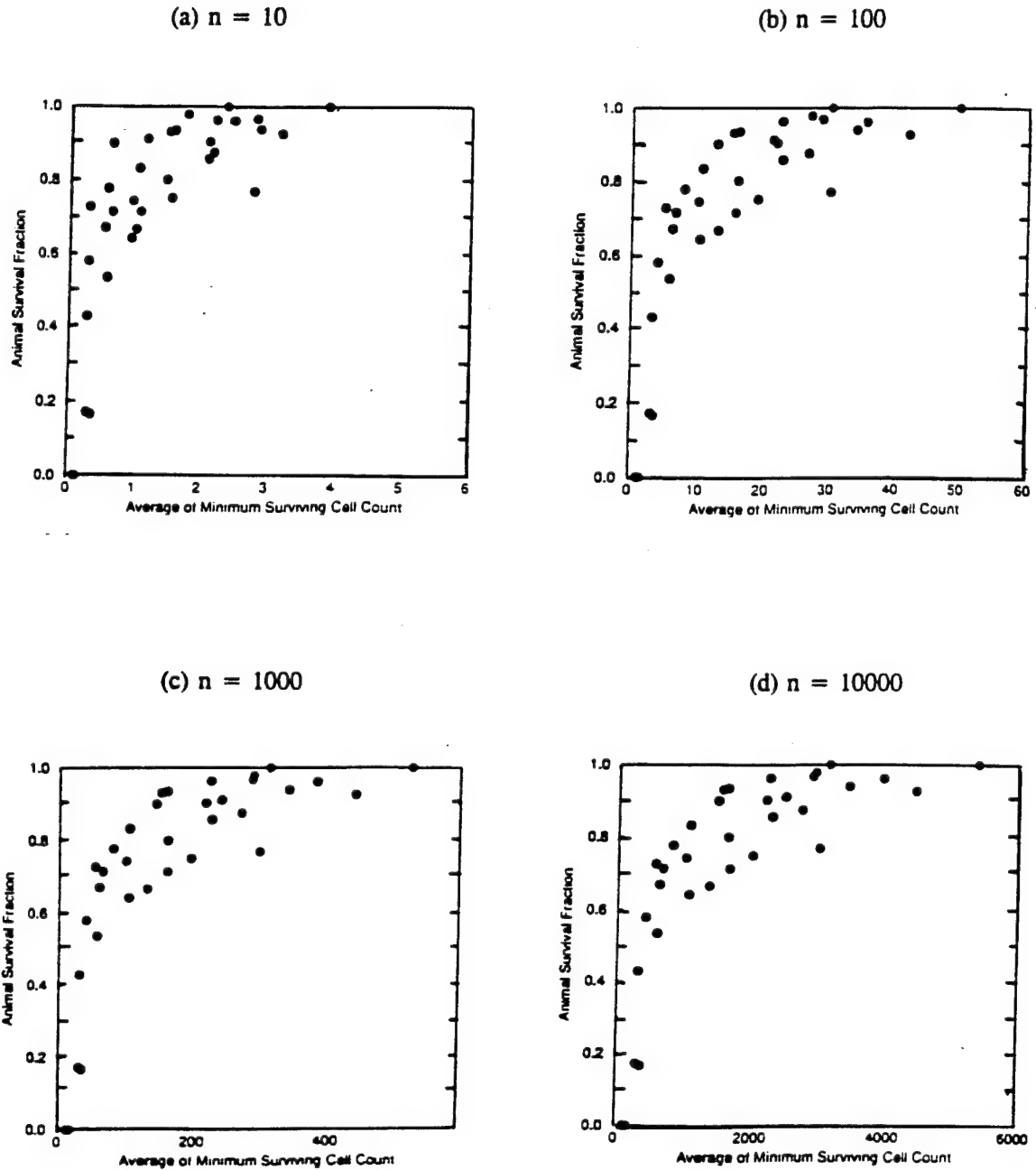


Figure 8-2. Cell survival vs animal mortality for  $n_0 = 10, 100, 1000$ , and  $10000$  in panels (a) through (d), respectively.

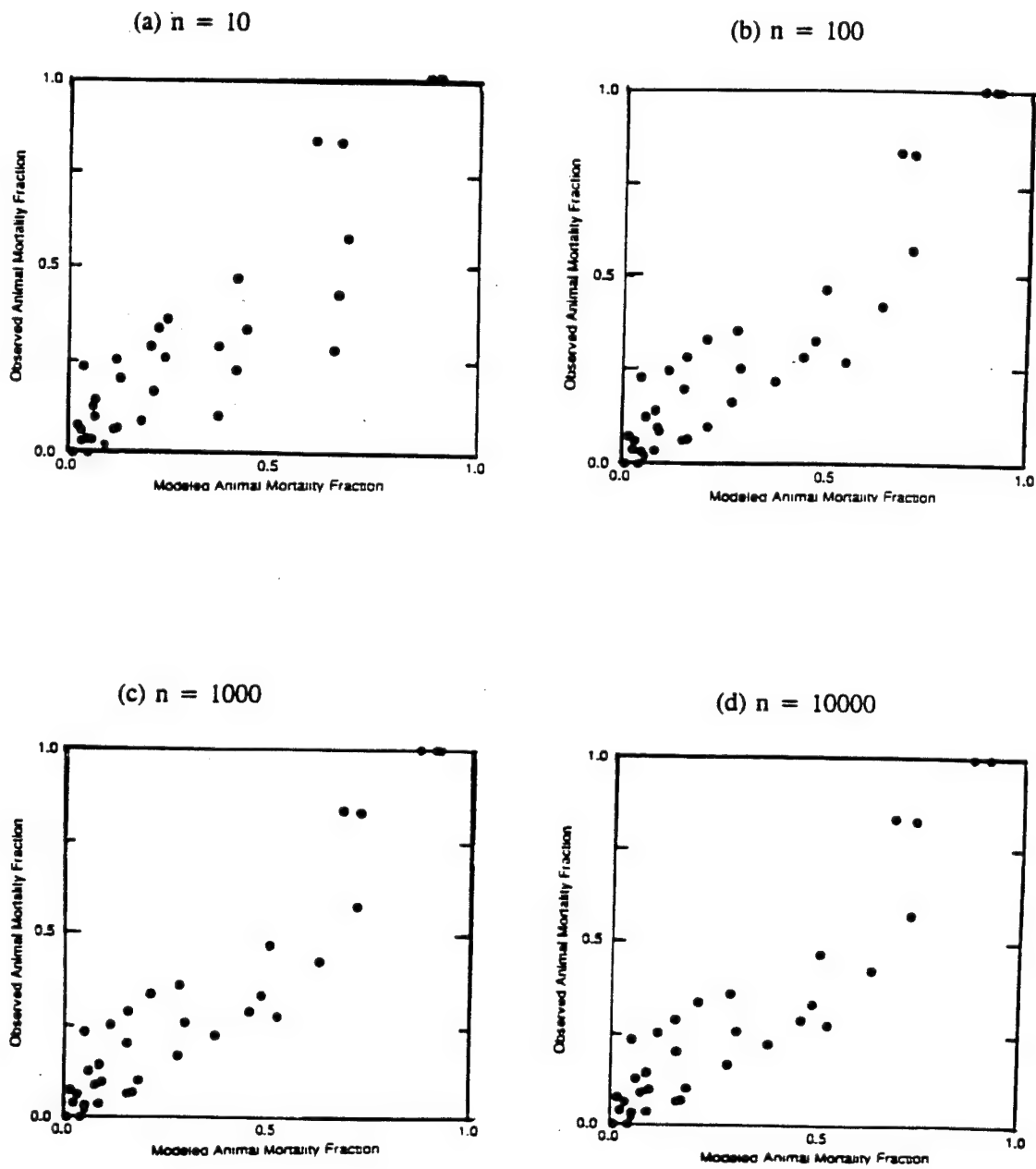


Figure 8-3. Modeled mortality vs experimental mortality for  $n_0 = 10, 100, 1000$ , and  $10000$  in panels (a) through (d), respectively.



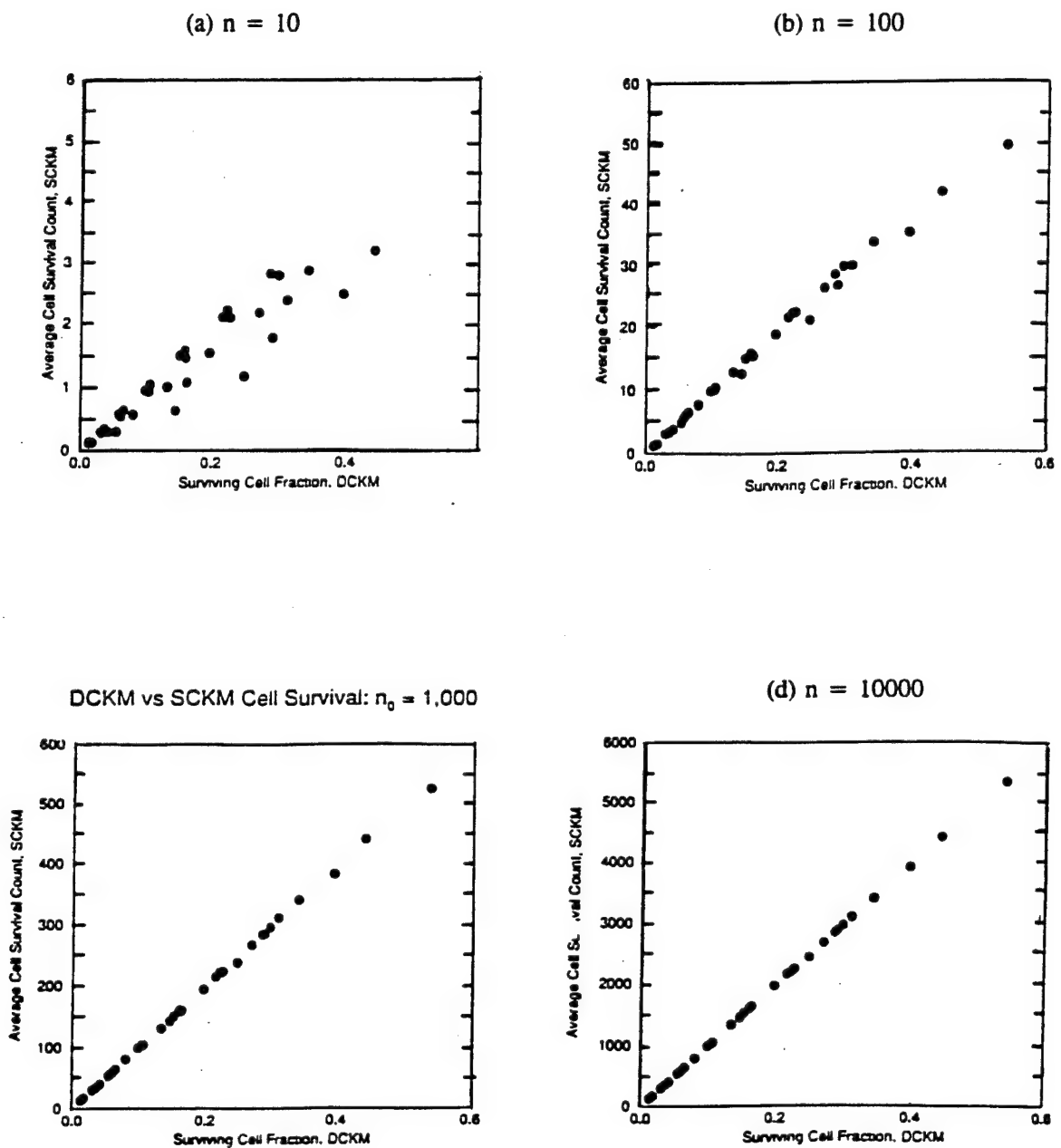


Figure 8-4. DCKM vs SCKM cell survival for  $n_0 = 10, 100, 1000$ , and  $10000$  in panels (a) through (d), respectively.

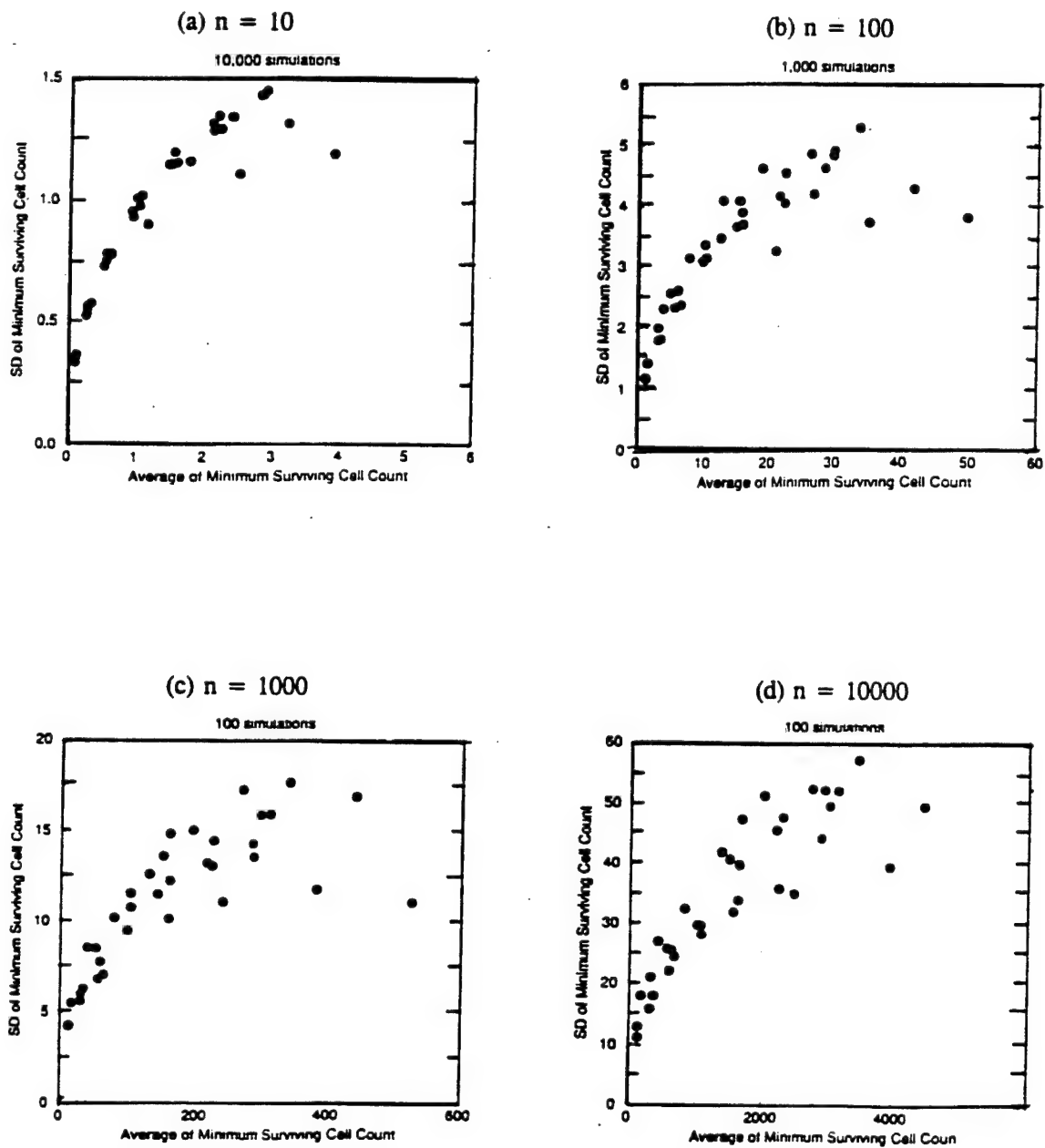


Figure 8-5. Average vs standard deviation for  $n_0 = 10, 100, 1000$ , and  $10000$  in panels (a) through (d), respectively.

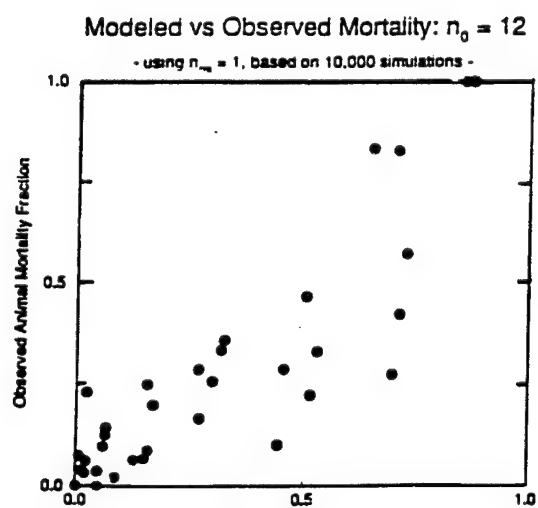


Figure 8-6. Modeled vs experimental mortality for  $n_0 = 12$ .

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APPENDIX  
ABBREVIATIONS

CFC = colony forming cell  
CFU-F = colony forming unit of marrow fibroblast  
CFU-S = colony forming unit in the spleen  
D = dose; D' = dose rate  
EPD = equivalent prompt dose of a radiation given in a pulse  
 $G_2$  = phase of the mitotic cycle  
GI = gastrointestinal  
Gy = unit of ionizing radiation  
 $LD_{50}$  = dose that is toxic to 50% of the test population  
M = median of the cumulative normal distribution (e.g.,  $LD_{50}$ )  
MLE = maximum likelihood estimation  
MSOD = many sets of data  
SSOD = single set of data  
S9 = enzyme extract used to activate compounds in test models  
S.D. = standard deviation  
 $\sigma$  = standard deviation of the normal distribution function  
 $T_D$  = doubling time  
X = value of abscissa

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